The National Eye Institute ANNUAL REPORT

FY 1977

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ANNUAL REPORT
NATIONAL EYE INSTITUTE
July 1, 1976 - September 30, 1977

STATEMENT OF THE INSTITUTE DIRECTOR

The National Eye Institute has just completed its seventh full year of operation. During the latest year, fiscal year 1977, a budget of $64 million was available. This is an increase of 30 percent over the previous year and almost three times the first NEI budget in FY 1970. The extramural budget alone grew $12 million during FY 1977 to more than $54 million. Research supported by these funds is directed toward reducing the personal and financial hardships caused by blindness and visual deprivation through improving our ability to prevent, diagnose, and treat blinding and disabling eye disorders.

Each year more than 4 million new cases of eye disease occur in the United States alone, necessitating more than 31 million yearly visits to eye specialists. An estimated 567,000 Americans are discharged from hospitals each year with an eye disease or injury listed as their principal diagnosis. The cost of such care, when added to the economic cost of blindness, exceeds $5 billion annually, according to estimates made in 1972; the cost today is probably at least 40 to 50 percent greater.

One of the leading causes of new adult blindness each year is diabetic retinopathy. During FY 1977 the NEI continued to support the nationwide clinical trial on diabetic retinopathy which reported the first conclusive evidence that photocoagulation, treatment with powerful beams of focused light, can reduce the rate of blindness from this disease by more than 50 percent over a two year period. Subsequent to these findings, a major effort was made to disseminate this information to eye care specialists and the general public. As a result, these findings have already had a significant impact on the treatment of diabetic retinopathy in the United States and abroad. A major new clinical trial was launched in FY 1977 testing the use of aspirin and photocoagulation in the treatment of nonproliferative and very early proliferative retinopathy with special attention to the management of diabetic maculopathy. The new study will span approximately 10 years, will include about 20 to 25 centers, and is one of the most important initiatives that the NEI has undertaken in the past seven years. In the NEI-supported nationwide study of vitrectomy, the surgical technique which is capable of restoring vision to some diabetic retinopathy patients who are already blind, most of the 13 participating centers have already begun enrolling patients. The scientific support of these trials is primarily provided by the NEI's Office of Biometry and Epidemiology. This organization is emerging as one of the key contributors to biostatistics and epidemiology in the United States.

Discoveries have also been made in the laboratory about the ways in which diabetes affects the eye. Such findings may also help us understand the serious complications of diabetes which occur elsewhere in the body. These studies have continued and expanded during the past year. For example,

organic compounds called flavonoids have proved to be the most effective means yet found of slowing the development of diabetic cataracts in animal models. Their ability to inhibit the enzyme which causes such cataracts may also have important implications for basic studies of the effect of diabetes on the eye. The NEI will continue to support this research.

The NEI's intramural program has continued its modest growth stressing the quality of personnel recruited and innovative approaches in its research program. Research advances have been made by the intramural staff and noted in this report in the areas of retinal biochemistry, cataracts, and glaucoma. Experts in immunology, retinal diseases, biochemistry, and ophthalmic pathology have been added to the staff in the past year. Groundbreaking for the Ambulatory Care Research Facility, the new addition to the Clinical Center, began this spring, and construction is currently underway. This expansion of the Clinical Center will result in a considerable increase in the NEI facilities for outpatient research and thus presents exciting new opportunities for the intramural program. Additionally, several new laboratories were made available this year to the Laboratory of Vision Research as a result of the completion of the addition to Building 6.

About one-third of all office visits for professional eye care are for conditions affecting the cornea. ARA-A, the first new drug in 15 years to treat herpes simplex keratitis, was developed with NEI support and approved this year by the Food and Drug Administration. It is expected to provide a long-needed alternative to present herpes therapy which is often ineffective or causes undesirable side effects. During the next fiscal year, the search will continue for even more effective antiherpes drugs.

During the past year further program planning initiatives were undertaken. The National Advisory Eye Council's Program Planning Subcommittee met several times with groups of consultants, which numbered over 150 when totalled, in order to establish the current needs and opportunities in vision research. A three-volume report delineating recommended research goals for vision scientists for the next five years will be published early next fiscal year.

Also during the past year, the NEI held a series of workshops to focus attention on areas of research where special opportunities exist for concerted action. One of these was a meeting to encourage the application of new laboratory tests of visual function and performance to the clinical diagnosis of eye diseases which are difficult to diagnose. Another workshop, to explore the research needs and opportunities in rehabilitation of partially-sighted individuals, was held at the NEI.

These and other research projects, contracts, collaborative trials, and studies initiated, supported, or conducted by the NEI will make progress toward the goal of relieving the enormous personal and economic costs of eye disorders.

Carl Kupfer

Carl Kupfer, M.D.

INTRAMURAL RESEARCH

Clinical Branch

ANNUAL REPORT
NATIONAL EYE INSTITUTE
July 1, 1976 - September 30, 1977

REPORT OF THE CLINICAL DIRECTOR

Elmer J. Ballintine, M.D.

The primary mission of the NEI Clinical Branch is to conduct research related to those aspects of ocular disease which can be studied best in man. Such investigations must meet the same standards of scientific rigor and validity that apply to other biologic experiments and do so within the ethical and humanitarian constraints imposed by the fact that the subjects are people.

Each research plan is reviewed by a protocol review committee composed of representatives from the Clinical Branch, other parts of the NEI and NIH, and others who are not employees of NIH. The protocol may also be reviewed by the Clinical Review Committee of the Clinical Center's Medical Board. These reviews insure that adequate safeguards for rights and welfare of patients are maintained and that patients are fully informed about both risks and potential benefits of their participation. Patients are accepted only if referred by an ophthalmologist outside the Institute and only if the patient's condition is appropriate for study in an approved protocol.

One of the objectives of the Clinical Branch is to demonstrate that rigorous clinical trials of important medical hypotheses can be performed within a single institution with a staff of modest size. One example is the Clinical Branch's Urokinase Central Retinal Vein Occlusion Trial. In this study patients with occlusion of the central retinal vein are randomly assigned to treatment with either heparin, urokinase or conservative treatment. Both patients and examiners are masked so that the treatment assignment is not known when the post-treatment examinations are performed.

Formal protocols incorporating these principles either are in operation or are being reviewed for testing surgical procedures for relief of hemorrhagic glaucoma, surgical procedures for treatment of glaucoma after multiple surgical failures, and the effectiveness of early treatment of ocular hypertension in preventing progression to chronic simple glaucoma.

The dominant concern of the laboratories in the Clinical Branch is the study of manifestations of human eye disease. These studies may require extensive development of methods and demonstration of biochemical or physiologic mechanisms as preliminaries to the work in patients. Examples are studies with the Q-switched laser for the treatment of experimental glaucoma in monkey, and the development of anterior chamber fluorophotometry for measurement of aqueous humor production in man.

Other laboratory investigations use material obtained from surgical, autopsy, or blood specimens. Thus, surgical specimens from patients undergoing trabeculectomy furnish samples of glaucomatous trabecular meshwork for growth in tissue culture in an effort to discover a metabolic defect responsible for simple glaucoma. Vitreous cavity washings obtained when

vitrectomy is performed on patients with diabetic retinopathy are fractionated and assayed for the presence of vasoproliferative factors.

In collaboration with the Experimental Pathology Section of the NEI Laboratory of Vision Research, 226 eyes from the autopsy service of the Clinical Center were processed and examined histopathologically during the past year. Approximately 1,305 inpatients and 1,196 outpatient consultations were furnished for other Institutes at the Clinical Center and there were 1,663 outpatient visits during the year. There were 82 inpatient admissions, and 41 surgical operations were performed.

The Clinical Branch continued to cooperate with other NIH Institutes in the pursuit of unique research opportunities. The study of diabetic retinopathy among the Pima Indians in a project administered by the Epidemiology and Field Studies Branch of the National Institute of Arthritis, Metabolism, and Digestive Diseases was continued as was the study of microangiopathy among patients with acromegaly. The study of ocular metastasis in National Cancer Institute patients undergoing treatment of breast carcinomas continued.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00150-04 CB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Ocular Hypertension Study

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elmer J. Ballintine	M.D.	Clinical Director	CB NEI
Other:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB NEI
	Richard A. Stone	M.D.	Clinical Associate	CB NEI
	Richard Weiblinger	B.S.	Biologist	CB NEI

COOPERATING UNITS (if any)

Office of Biometry and Epidemiology, NEI

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with ocular hypertension are randomly assigned to treatment with topical pilocarpine in one or both eyes or to no treatment. The objectives of the study are: 1) to determine if treatment with pilocarpine to reduce intraocular pressure before visual field changes occur will reduce the number of ocular hypertensive subjects who eventually become glaucomatous, and 2) to determine if measurements of aqueous humor dynamics; the response to water loading of diurnal variation of intraocular pressure; serial stereophotographs of the optic disc, and measurements of visual fields help to predict which patients will eventually become glaucomatous.

Project Description:

Objectives: Prolonged observation of a series of patients with ocular hypertension, some of whom are treated with miotics, will help to determine which signs have value in predicting those who will eventually require treatment and in determining if early treatment of ocular hypertension has any value in preventing visual field loss or in slowing the rate of development of abnormalities of aqueous humor dynamics.

Methods Employed: A detailed plan for classifying patients with ocular hypertension, observing them by repeated examinations over a period of five or more years, and randomly assigning patients to treatment with pilocarpine collyria in one or both eyes, or to no treatment, has been standardized for repeated measurement of visual fields, aqueous humor dynamics, and photogrammetry of the optic discs.

Major Findings: The protocol for conduct of the study has been completed and recruitment of patients in the study is continuing. Thirty-eight patients have been enrolled.

Significance to Biomedical Research and the Program of the Institute: Early, precise identification of patients who require treatment because they are in the early stages of the simple glaucoma remains an unsolved problem. The data being collected on this research plan will furnish a basis for establishing criteria for treatment more precisely than is now possible. There is at present no detailed knowledge of the progression of optic disc changes in ocular hypertension. The data being collected in this study, as well as the development of better instruments for the measurements in this study, will supply needed information in this field.

Proposed Course: It is expected that the project will continue for at least five years, and we expect to enroll 100 subjects.

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: Human

Research Objective: Diagnosis, Treatment

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00017-03 CB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Tissue Culture of Trabecular Meshwork

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Elmer J. Ballintine	M.D.	Clinical Director	CB NEI
Other:	Richard A. Stone	M.D.	Clinical Associate	CB NEI
	Richard Weiblinger	B.S.	Biologist	CB NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1	0.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Slices of trabecular meshwork from normal monkey eyes and from surgical trabeculectomy specimens from human glaucomatous eyes are being grown in tissue culture. Attempts are being made to identify the tissue of origin of the resulting cellular growth, and efforts are being made to selectively grow trabecular endothelial cells.

Project Description:

Objectives: Much evidence indicates that in simple open-angle glaucoma the obstruction to aqueous humor outflow lies within the trabecular meshwork and inner wall of Schlemm's canal. The amounts of human trabecular tissue available for biochemical and physiologic study are insufficient for most in vitro research methods. Therefore, tissue culture techniques are being employed in the hope of developing a system in which the basic physiologic and biochemical abnormality present in open-angle glaucoma can be explored. After a satisfactory culture system is developed, various metabolic and physiologic parameters of the cultured cells will be explored.

Methods Employed: Specimens of trabecular tissue are obtained from monkey eyes for preliminary studies. Some surgical specimens from patients undergoing trabeculectomy have been studied. More of these surgical specimens as well as controls from human autopsy eyes are being sought.

Specimens of trabecular meshwork are sectioned into small fragments under the dissecting microscope and placed in tissue culture medium. Phase contrast microscopy is used to observe growth and form of these cells. They are being further characterized by their histologic and histochemical properties. Methods and criteria for growing trabecular epithelial cells free of fibroblasts are being developed.

Major Findings: Trabecular meshwork from monkey and human eyes has been grown consistently in tissue culture, and the conditions for this growth have been determined. It has been possible to obtain some cultures without a significant fibroblastic contamination.

Significance to Biomedical Research and the Program of the Institute: The mechanism by which the resistance to aqueous humor outflow increases in open-angle glaucoma is at present unknown. This project may be able to define the physiologic and biochemical abnormalities of trabecular epithelium that are the fundamental causes of open-angle glaucoma.

Proposed Course: After further refining of the tissue culture technique, metabolic studies of the cultured cells will attempt to demonstrate differences between normal cultures and those from human eyes that have a pressure elevation following topical corticosteroids.

NEI Research Program: Glaucoma - Etiology of Glaucoma (Primary Glaucoma/Open-Angle Glaucoma)

Experimental Subject or Tissue Source: Monkey/Human

Research Objective: Etiology

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00054-01 CB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cornea: Organ and Cellular Cultures of Epithelium Stroma and Endothelium.

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David BenEzra	M.D.	Visiting Scientist	CB NEI
Other:	Teuro Tanishima	M.D.	Visiting Scientist	CB NEI
	Sandra Bornstein		Technician	CB NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.5	0.5

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The metabolic activity of cells from the various layers of the cornea and the possible in vitro regulatory mechanism between the layers were investigated: 1. A microculture for accurate and reproducible information concerning the rate of DNA of protein synthesis at a cellular level has been developed. 2. Organ cultures of endothelium with its Descemet's membrane were used as a model for the study of endothelial wound repair mechanism in vitro. The possibility of transplantation of these organ cultures was also evaluated. 3. Combined organ cultures: The effects of the various layers of the cornea on each other were studied in mixed organ cultures. A possible interaction and/or regulation between epithelium and stroma has been observed. Studies are underway in order to detect any possible interactions in stroma-endothelium or endothelium-epithelium organ cultures.

Project Description:

Objectives: This study focused on the following: 1) Investigate the metabolic activity of corneal epithelium, stroma and endothelium at a cellular level. Develop a method for the accurate assessment of the effects of drugs on the various layers of the cornea in normal conditions and in diseased states 2) Develop an in vitro model for the study of the endothelial wound healing mechanism. Test the possibility of organ transplantation of cultured endothelium instead of transplantation of a whole cornea. 3) Study a possible existing regulatory mechanism between the various layers of the cornea.

Methods Employed: Corneas (guinea pig, rabbit, monkey) are removed and dissected under an operating microscope.

Macrocultures

These are initiated in petri dishes (Falcon 3001). Explants of tissues from corneas are covered with a glass cover-slip and a drop of culture medium (RPMI 1640 with 5 or 10% serum) is allowed to diffuse under it. Cultures are incubated overnight at 37° in 100% humidity atmosphere and a continuous flow of 5% CO_2 and 95% air. After 16 hours of incubation, one milliliter of medium is added and the cultures further incubated as above. On the third day the coverslips are removed and 0.5 ml of fresh medium added. Culture medium is then changed every four days.

Microcultures

On the 8th to 10th day, macrocultures are subjected to trypsinization (0.25% trypsin and 0.05% EDTA), cells counted and microcultures initiated in microtiter plates (Falcon 3040). DNA and protein synthesis are evaluated by the extent of tritiated thymidine or tritiated amino acids uptake. Transmission and scanning electron microscopy was carried out at different intervals after initiation of the macrocultures. The original explants as well as the patterns observed among the outgrowing cells were studied.

Major Findings: a) Accurate and reproducible information concerning the metabolic activity of cells from the different layers of the cornea could be obtained with 10^3 cells per microculture. The origin and concentration of the added serum in the medium influenced markedly the metabolic activity of all corneal cells tested. Cultures without serum did not demonstrate any active DNA or protein synthesis but excluded trypan blue. Addition of 1% serum boosted the DNA and protein synthesis. Xenogeneic serum had the most potent stimulatory effect on both activities. Allogeneic and autologous sera were less stimulatory as compared to the xenogeneic serum. However, both allogeneic and autologous sera stimulated relatively more protein synthesis than DNA synthesis while xenogeneic serum had a stronger stimulatory effect on the DNA synthesis relative to protein synthesis. The epithelial and stromal cells showed similar patterns of metabolic activity. The endothelial cells in this system demonstrated a different pattern of behavior. These differences are now being further investigated to lessen the effect of drugs on the various layers of the cornea.

Endothelial wound healing

b) Isolated Descemet's membrane with endothelium was kept in vitro and followed up. Morphological changes in the culture were recorded at intervals by light, scanning and transmission electron microscopy. It was found that corneal endothelial cultures demonstrate a "repair" activity near wounds as early as one hour after the initiation of culture. The endothelial cells neighboring wounds round-up, elongate and mimic fibroblast-like structure. Most of the activity is observed around the edges of the wound while the advancing cells get bigger and move toward the center of the wound. In the process of "filling the gap" active cells revert to a morphological appearance characteristic of the endothelial cells. In rabbit endothelial cultures, "overzealous" cells proliferate and override the other endothelial cells after healing of the wound is complete. In contrast, guinea pig and monkey endothelial cultures showed a regular monolayer pattern. c) Electron microscopic studies of pure epithelial and stromal cultures on one hand and of combined cultures on the other hand demonstrated that keratocyte activity was inhibited by the presence of epithelial cells in culture. Pure culture of stromal cell showed a collagenolytic activity inside the explant. The collagenolytic activity of the keratocytes is not detectable when epithelial cells are present on the explant. Furthermore, using the microculture method it was demonstrated that supernatants of epithelial cell cultures had a marked inhibitory effect on the keratocytes capacity to synthesize DNA. No effect of stromal cell supernatant on epithelial cell activity could be detected by the same methods.

Significance to Biomedical Research and the Program of the Institute: The obtained data and those expected from future investigations would help in understanding the repair mechanism of corneal injuries as well as the pathology of dystrophies and inherited disorders of the cornea. The possibility of transplanting cultured endothelium would have a great impact on transplantation of the cornea.

Proposed Course: 1) Further investigate on the transplantability of the endothelial cultures. 2) Elucidate other possible regulatory influences between stroma and endothelium and between the latter and epithelium.

NEI Research Program: Corneal Diseases - Corneal Transplantation and Stromal Injury and Repair/Corneal Edema, Dystrophies, and Inherited Disorders.

Experimental Subject or Tissue Source: Rhesus monkey/Rabbit/Guinea pig.

Research Objective: Etiology, Treatment

Publications:

BenEzra, D.: A micro culture technique for the evaluation of corneal cell metabolism in vitro. Invest. Ophthalmol. (in press).

BenEzra, D.: A in vitro model for the study of corneal endothelial repair. Tenth Annual Research Conference, Boston 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00038-05 CB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Studies of Choroidal-Retinal Degenerative Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Donald R. Bergsma	M.D.	Senior Staff Ophthalmologist CB NEI
Other:	Muriel Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist CB NEI
	A. Ralph Rosenthal	M.D.	Chairman, Department of Ophthalmology, Stanford University
	David Huxal	M.D.	Deputy Director, Veterinarian Medicine, Walter Reed Army Institute of Research, Washington, D.C.

COOPERATING UNITS (if any)
Walter Reed Army Institute of Research (Division of Surgery). Washington, D.C.

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.50	0.50	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☒ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The long range purposes of this project are (1) to improve clinical classification of selected degenerative diseases of the retina and choroid by means of detailed, longitudinal studies of retinal function and clinical laboratory tests, and (2) to provide a clinical resource for related laboratory investigations of the cause, prevention and therapy of these diseases. The degenerations of the eye such as retinitis pigmentosa are often of familial or genetic origin and must be distinguished from nonprogressive forms of night blindness and from drug or other toxicity. Degeneration of the macula also occurs in some of these diseases. Animal models are used for related investigations of retinal functions, degenerations and toxicity. Serum, biopsy and autopsy specimens available because of this project have been studied in the laboratory via project Z01 EY 00134-03 LVR (biochemical and tissue culture studies).

Project Description:

Objectives: The objectives of this study are (1) to improve clinical classification of selected degenerative diseases of the retina and choroid, and (2) to provide a clinical resource for related laboratory investigations of the cause, prevention and therapy of these diseases. Examples are retinitis pigmentosa, familial macular degeneration, and the effects of drugs toxic to the retina.

Methods Employed: Clinical studies utilize specialized tests of visual function (dark adaptation, cone thresholds, visual fields), electroretinography (ERG), electro-oculography (EOG), fundus photography and fluorescein dye studies. Appropriate testing of relatives is undertaken to document genetic patterns and define variation of severity within disease entitites. Observation of monkeys poisoned with chloroquine is continuing. Patients with retinal toxicity due to drugs such as chloroquine and thioridizine are being studied.

Major Findings: Approximately 100 patients were studied this year. At present, the overwhelming majority of patients afflicted with choroidal and retinal degenerative diseases are not curable. No proposed therapy shows enough promise to warrant a treatment trial. Nevertheless, most patients are helped by a combination of genetic counselling, discussing of prognosis and advice regarding visual aids and rehabilitation. Moreover, serum, biopsy and autopsy specimens available because of this project have been studied biochemically and pathologically via related laboratory projects such as Z01 EY 00134-03 LVR. This led to a combined electronmicroscopic and biochemical study of autopsy eyes with retinitis pigmentosa which showed that one of the two normal receptors for vitamin A is absent in the late RP retina.

Related experiments in monkeys show that the subcellular damage to retinal cells produced by high doses of chloroquine occurs more than four years before ERG or fundus abnormalities are detectable.

Significance to Biomedical Research and the Program of the Institute: This project is directed at improving classification, prevention, and treatment of choroidal-retinal degenerative diseases via new diagnostic techniques, controlled therapeutic trials, long-term follow-up, and animal and laboratory experimentation.

Proposed Course: Because an adequate panel of patients has been established for longitudinal studies, special laboratory investigation, and, when appropriate, therapeutic trials, emphasis has been shifted to related laboratory studies via project Z01 EY 00134-03 LVR. Heterogeneity within clinically defined groups hinders this research. Therefore, emphasis is now on establishing subpanels with adequate criteria to insure homogeneity.

NEI Research Program: Retinal and Choroidal Diseases-Developmental and Hereditary Disorders

Experimental Subject or Tissue Source: Human/Rhesus monkey

Project No. Z01 EY 00038-05 CB

Research Objective: Etiology, Diagnosis, Treatment

Publications:

Bergsma, D.R., Wiggert, B., Funahashi, M. Kuwabara, T., and Chader, G.: Vitamin A receptor in normal and dystrophic human retina. Nature 265: 66-67, 1977.

Bergsma, D.R., Funashashi, M., Kuwabara, T., and Christiansen, J.M.: Ultramicroscopic and vitamin A receptor abnormalities in retinitis pigmentosa at autopsy. Trans. Am. Acad. Ophthalmol. (in press).

Bergsma, D.R.: The Usher syndrome: Clinical definition and related research, In Proceedings of the Usher Syndrome Conference, Helen Keller National Center for the Deaf-Blind, Port Washington, NY, Dec. 1-3, 1976 (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00058-01 CB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Suppressor Lymphokines in an In Vitro System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jane Blackman	M.D.	Senior Staff Fellow	CB NEI
Other:	Joost Oppenheim	M.D.	Chief	LMI NIDR
	Terrell Hoffeld	D.D.S, Ph.D.		LMI NIDR

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

We are investigating the relationship of suppressor and helper lymphokines in several in vitro systems in mice and rabbits. We have developed an assay system for suppression of B cell proliferation. We plan to characterize a suppressor of B cell proliferation with the eventual plan of testing this in a rabbit experimental uveitis model.

Project Description:

Objectives: We are investigating lymphokines and their interrelationships in the mechanism of cellular immunity. Past studies of uveitis have placed much emphasis on humoral immunity, but most humoral immunity appears to be modulated by cellular immunity. Of recent interest are lymphokines, i.e. proteins released by lymphocytes or macrophages which regulate the immune responses of cellular proliferation, cellular motility, antibody synthesis, or enzyme manufacture and release.

Therefore, to study suppressor lymphokines or regulator lymphokines would help to elucidate the mechanisms of inflammation in the eye and possible treatment. The immediate goal is to characterize a lymphokine suppressor of B^- cell proliferation and investigate its relationships with other B cell functions or other B cell regulators. A long term goal is to use this suppressor or regulator lymphokine in an experimental ocular inflammation model. Eventually it is hoped that this can be used therapeutically in uveitis in human beings.

Methods Employed: An in vitro assay system of suppression of B cell proliferation has been developed. We have stimulated mouse B lymphocytes in vitro with endoxtoxion. Consequently, B cells incorporate tritiated thymidine into their DNA as a signal of proliferation. A suppressor lymphokine has been made in a manner similar to that described by Rich and Pierce, to which they have given the name Soluble Immune Response Suppressor (J. Immunol. 112:1360, 1974). This involves stimulating mouse spleen cells in vitro with the T cell mitogen Concanavalin A and harvesting the supernatant. The in vitro assay system for thymocyte or T cell proliferation and the assay system of Jerne plaques for antibody synthesis are also being studied to learn the relationship of suppressor lymphokines to other immune mechanisms.

We plan to fractionate and characterize crude supernatants which contain this suppressor of antibody synthesis. This suppressor probably does not cross species and does not affect T cell proliferation by a mitogeneic factor, also present in the crude supernatant.

Major Findings: An assay system for B cell suppression in vitro has been developed.

Significance to Biomedical Research and the Program of the Institute: Lymphokines as a part of the immune response in inflammatory diseases of the eye, especially uveitis, have recently begun to be investigated. They probably have a very important role in initiating, enhancing, and suppressing the reactions seen clinically. We wish to pursue their possible role in the breakdown of different immunologic reactions by separate compartments of the eye and the regulations of acute or chronic uveitis.

The Eye Institute is interested in elucidating and controlling the mechanisms of inflammatory ocular diseases, whether of corneal, uveal or retina origin.

Proposed Course: The first step is to fractionate crude supernatant made from Con A stimulated spleen cells. This will begin to characterize the suppressor lymphokine. Then other definitive tests can be peformed to more completely characterize this material.

After this the suppressor lymphokine can be tested for its relationship to other immune systems e.g. for its ability to regulate the functions of macrophages to make lymphokines, to show motility or to phagocytose foreign materials. The suppressor lymphokine will be tested for its regulation of other lymphokine functions or synthesis.

The projected study is then to make this suppressor substance in other animal systems, the rabbit or guinea pig initially. In an experimental uveitis model this material then can be tested for its ability to suppress or regulate a response.

NEI Research Program: Retinal and Choroidal Diseases - Inflammatory Disorders

Experimental Subject or Tissue Source: Mouse

Research Objective: Treatment

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00020-03 CB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Parametric Studies of Eye Movement Disorders in Humans

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David G. Cogan	M.D.	Staff Ophthalmologist	CB NEI
Other:	John Gittinger	M.D.	Clinical Associate	CB NEI
	Fred C. Chu	M.D.	Senior Staff Fellow	CB NEI
	David S. Zee	M.D.	Consultant	CB NEI
	Douglas B. Reingold	M.A.	Research Associate	CB NEI

COOPERATING UNITS (if any)

Experimental Therapeutics Branch, NINCDS

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	2.0	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Our intention in this work is to identify and measure the ocular motor disabilities that accompany each of several neurological diseases. We have developed tests of ocular motor subsystems which can reveal these disorders, their progress and their response to therapy; advanced our understanding of the mechanisms of these diseases; and gained insight into the roles of some brain structures in the control of eye movements. We are monitoring electro-oculographically the fixation, saccade, pursuit, optokinetic and vestibular system, estimating on a laboratory digital computer the parameters of these models under normal and pathological conditions. The disorders under current study are: 1) cerebellar tumors and degenerative diseases; 2) Parkinson's disease; 3) progressive supranuclear palsy; 4) parietal lobe lesions; and 5) downbeat nystagmus. In addition we are observing the oculomotor changes accompanying normal aging.

Project Description:

Objectives: To identify and measure the ocular motor disorders in several diseases and to elucidate their pathophysiology.

Methods Employed: We test ocular motility using visual and vestibular stimuli, the former consisting of fixation targets and discreet moving targets presented on a video monitor, and a full-field optokinetic drum surrounding the patient. Vestibular stimuli consist of rotations of the seated patient about a vertical axis, by means of a precision servo-motor. Eye movements are monitored using electro-oculography and analyzed by laboratory digital computer to yield estimates of eye velocities and oculomotor system gains, latencies, and time constants.

Major Findings: In cerebellar disease, disorders of the saccadic, pursuit, fixation, optokinetic, and vestibular subsystems have been identified. The cerebellum is involved in the maintenance of eccentric gaze, the production of smooth pursuit eye movements, control of the amplitude of saccadic eye movements, and the interaction of visual and vestibular eye movement reflexes. We have designed a test which seems to be quite specific for cerebellar disease in which the patient is asked to fixate a target which remains in front of him while he is rotating. Analysis of the resulting involuntary eye movements provides a measure of visual-vestibular interaction which may correlate with the extent of damage to the cerebellum.

A defect in the optokinetic system of subjects with loss of labyrinthine function has been observed. This implies that brain structures involved in the production of optokinetic nystagmus are subject to and dependent upon vestibular influences, and accords well with the concept of the optokinetic system's being distinct from the pursuit system in that it contributes to self-motion perception considerably more than the pursuit system does.

Abnormal saccades have been observed in a group of patients with monocular impairment of extraocular muscle function. When refixating in the direction opposite to the paralysis with the paretic eye, a series of saccades is made with the eye drifting back to the starting position between each saccade. This is consistent with the assumption that the estimate of eye position that the central nervous system uses for the control of saccadic eye movements is based upon efferent rather than afferent information.

Significance to Biomedical Research and the Program of the Institute: Tests have been created which can indicate the presence and the severity of oculomotor involvement in the diseases studied. The mechanisms of the oculomotor deficits in these diseases have been clarified, and evidence has been put forward on the roles of several brain structures in the control of eye movement.

Proposed Course: This project will be continued.

NEI Research Program: Sensory and Motor Disorders of Vision

Project No. Z01 EY 00020-03 CB

Experimental Subject or Tissue Source: Human

Research Objective: Etiology

Publications:

Cogan, D.G., Yee, R.D., and Gittinger, J.: Rapid eye movements in myasthenia gravis. Arch. Ophthalmol. 94: 1083, 1976.

Yee, R.D., Cogan, D.G., and Zee, D.S.: Ophthalmoplegia and dissociated nystagmus in abetalipoproteinemia. Arch. Ophthalmol. 94: 571, 1976.

Yee, R.D., Zee, D.S., and Cogan, D.G.: Congenital ocular motor apraxia. Brain. (in press).

Yee, R.D., Cogan, D.G., Zee, D.S., Baloh, R.W., and Honrubia, V.: Rapid eye movements in myasthenia gravis II. Electro-oculographic analysis. Arch. Ophthalmol. 94: 1465, 1976.

Cogan, D.G.: Opsoclonus. Handbook of Clinical Neurology. Vol. 33, No. Holland Publishing Co. (in press).

Cogan, D.G., Robins, S.M.: Neuro-ophthalmic disorders. Human Health and Disease. Biological Handbooks II: 288-293. Fed. of Amer. Soc. for Exp. Biol., Bethesda, MD.

Cogan, D.G.: Enigmas and hypotheses. ARVO Symposium on Eye Movements, San Antonio, Texas. Oct. 26, 1976. (in press).

Zee, D.S., Yee, R.D.: Abnormal saccades in paralytic strabismus. Am. J. Ophthalmol. 83: 112, 1977.

Zee, D.S., Yee, R.D., Cogan, D.G., Robinson, D.A., and Engel, W.K.: Ocular motor abnormalities in hereditary cerebellar ataxia. Brain 99: 207, 1976.

Zee, D.S., Yee, R.D., and Robinson, D.A.: Optokinetic responses in labyrinth-defective human beings. Brain Res. 113: 423, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 000154-04 CB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Experimental Glaucoma in the Rhesus Monkey

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB NEI
Other:	Teruo Tanishima	M.D.	Visiting Scientist	CB NEI
	Helen MacLellan	M.S.	Biologist	CB NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.6	0.2	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this investigation is to study the morphology, the physiologic function, and the pharmacologic behavior of the eye of the rhesus monkey in its normal state compared to its state when experimental glaucoma has been induced by argon laser photocoagulation of the trabecular meshwork. Autoradiographic studies of axoplasmic transport in chronic experimental glaucoma have been completed. Currently the effects of intraocular pressure elevation on optic nerve and retina are being evaluated by electron microscopic examination of the tissue and preparation of whole-mount retinas.

Project Description:

Objectives: To study physiologic function, pharmacologic behavior, and morphology of the monkey eye after induction of glaucoma by argon laser photocoagulation of the trabecular meshwork. To compare observations to control normal eyes.

Methods Employed: Circumferential argon laser photocoagulation of the rhesus monkey trabecular meshwork eventually causes sustained elevation of intraocular pressure to the range of 30 to 55 mmHg, the pressure range found in many patients with open-angle glaucoma. This is in contrast to the acute, short duration, very high pressure elevation (more than 65 mmHg, up to 95 mmHg) seen in most models for glaucoma. Outflow facility is evaluated by perfusion. Aqueous flow is determined by turnover of radioiodinated serum albumin injected into the anterior chamber. Retinal and optic nerve function can be studied by autoradiography and electron microscopy to evaluate morphologic evidence of altered axoplasmic flow. The retina can also be studied in cross section or by preparing whole mounts of the tissue.

Major Findings: In FY 1977, histology and autoradiography of retina and optic nerve were studied. Data were obtained from 18 eyes of 9 monkeys. There were 10 eyes with experimental glaucoma, 7 untreated control eyes, and 1 eye which did not have glaucoma but which had received laser treatment of the angle structures (treated control). These eyes are important because they are the first with sustained elevation of intraocular pressure in the 35 to 50 mmHg range to be studied for pressure effects on axoplasmic flow. Previous studies have been performed by other investigators in monkey eyes with acute elevations of intraocular pressure to the range of 65 to 95 mmHg in most cases. This is higher and of shorter duration than the findings in chronic open-angle glaucoma patients.

In our experiment the autoradiographs were difficult to interpret. There was considerable variation in grain density from slide to slide for the same eye, and there was sparsity of labelling. A preliminary impression is that the glaucoma eyes and the control eyes did not show obstruction of axoplasmic transport in a way similar to eyes with acute, very high elevation of intraocular pressure. There are several possible explanations. First, intravitreal injection of the radioactive label produced a lowering of intraocular pressure in the interval before enucleation (from 14 to 48 hours). This lowering of pressure might have released an obstruction of axoplasmic flow. Second, it is possible that changes in axoplasmic flow in chronic mild to moderate glaucoma are too subtle to be detected by light microscopic, autoradiographic technique

For these reasons, a study was initiated employing an electron microscopic examination of glaucomatous monkey eyes. This will eliminate the artificial reduction in intraocular pressure at the time of sacrifice caused by intravitreal injection, and furnish more sensitive information on changes in the retina, nervehead, and optic nerve. To date two monkeys with monocular glaucoma (IOP greater than 30 mmHg for at least 3 weeks) have been enucleated for electron microscopic evaluation of the retina, optic nervehead, and optic nerve. Four other monkeys have received laser treatment to one or both eyes

and their intraocular pressure changes are being monitored. Results are not yet available. Plans are to continue the project.

We are also developing a technique for preparing whole mounts of monkey retinas. We shall evaluate the distribution and density of the various cell populations in the normal retina and compare this to the retina in eyes with chronic experimental glaucoma with mild to moderate cupping of the optic nervehead.

Significance to Biomedical Research and the Program of the Institute: All parts of the project are immediately related to clinical problems in glaucoma. This experimental glaucoma is the best model available for human chronic open-angle ("simple") glaucoma. Using this model allows close examination of the retina and optic nerve with the promise of additional insight into the mechanism of loss of visual function in the patient with glaucoma.

Proposed Course: The project will continue.

NEI Research Program: Glaucoma - Primary Glaucoma--Open-Angle Glaucoma/ Secondary Glaucoma

Experimental Subject or Tissue Source: Rhesus monkey

Research Objective: Etiology

Publications:

Gaasterland, D.E.: Axoplasmic transport in eyes with chronic elevation of intraocular pressure. Presented at the National Eye Institute Symposium on Experimental Eye Pathology, October 14, 1976, NIH, Bethesda, Maryland 20014.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00168-02 CB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Laser Surgery for Glaucoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB NEI
Other:	Charles Bonney	D.V.M., Ph.D.	Visiting Scientist	CB NEI
	Elmer J. Ballintine	M.D.	Clinical Director	CB NEI
	Carl Kupfer	M.D.	Director	NEI
	Thomas Clem		Electronics Engineer	BEIB DRS
	Harold W. Tipton		Mechanical Fabrication	BEIB DRS

COOPERATING UNITS (if any)

Biomedical Engineering and Instrumentation Branch, Division of Research Services, NIH; Armed Forces Radiobiology Research Institute

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.3	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The high energy and power that are found in some laser beams offer a tool for noninvasive alteration of anterior intraocular tissue. Specifically, iridotomy and trabeculotomy are possible. Such surgery has importance for glaucoma patients because of potential improvement of favorable surgical outcome statistics and reduced surgical morbidity. This project has as its aim a systematic evaluation in simian (rhesus) eyes of laser effects and the application of promising systems and procedures to human glaucoma eyes under controlled conditions. Current work is directed to obtaining a proper Q-switched ruby laser system and to initial testing in monkey eyes.

Project Descrpition:

Objectives: To develop workable laser systems for anterior segment surgery and to apply the systems to the normal monkey eye. To study the physiologic and morphologic effects of laser energy upon monkey eyes. To apply favorable systems to the glaucoma eye of humans under controlled conditions.

Methods Employed: In this, the second year of this project instrument development and elimination of mechanical and electronics problems have continued to require considerable amounts of time. Tissue studies for morphology and for physiologic function are done with standard methods: perfusion of the anterior chamber to determining outflow facility; turnover of RISA to determine flow; and gross, light and electron microscopic tissue examination.

Major Findings: Two monkeys have been irradiated in vivo, and a number of monkey eyes have been irradiated in vitro. In one monkey, irradiation of the trabecular meshwork area resulted in considerable bleeding associated with a large cyclodialysis. In the other in vivo study, irradiation of the iris easily produced an iridotomy. Current problems center around continued difficulty with the viewing optics and difficulty with knowing the energy content of the flash of light delivered to the eye.

Dr. C. Bonney has joined the project as an investigator. His Air Force experience with laser effects upon the retina and with lasers in general contribute to solution of problems of instrument development; his veterinary medicine training contribute to the skills available for the animal studies.

Significance to Biomedical Research and the Program of the Institute: Potentially, a physically noninvasive laser system for anterior segment surgery might replace conventional invasive operative procedures for several types of glaucoma. This possibility is in its infancy at this time.

Proposed Course: The project will be continued.

NEI Research Program: Glaucoma - Etiology of Glaucoma (Primary Glaucoma--Open-Angle Glaucoma/Primary Glaucoma--Angle-Closure Glaucoma/Secondary Glaucomas)

Experimental Subject or Tissue Source: Human/Monkey

Research Objective: Treatment

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00143-04 CB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Radioiodinated Chloroquine Analog for Diagnosis of Ocular Melanoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB NEI
Other:	Elmer J. Ballintine	M.D.	Clinical Director	CB NEI
	Carl Kupfer	M.D.	Director	NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this clinical investigation is to assess the value of systemically administered I-125 labelled chloroquine analog for the detection of ocular melanoma. Patient enrollment terminated 30 June 75, after the 36th patient was accepted. Current interest is in continued follow-up examination of the patients. This will yield information regarding the clinical course of diagnosed and treated melanoma patients, of diagnosed melanoma patients who refused treatment, and of patients with lesions which may or may not be ocular melanoma. The course will be compared to the results of the radioactive tracer testing.

PHS-6040

Project Description:

Objectives: To determine the value of using I-125 labelled chloroquine analog for the detection of ocular melanoma.

Methods Employed: During this year a number of standard follow-up clinical examinations have been performed.

Major Findings: In one patient with a negative test in 1975, tumor growth was documented this year and enucleation advised. This was performed at the National Naval Medical Center. Histologic examination confirms the lesion to be a spindle B type melanoma. Two additional patients with highly suspicious lesions for clinical reasons have had documentation of tumor growth during the year. Both continue to refuse enucleation. Of these two, one had a positive and one a negative test in 1975. One patient with a positive test in 1974 continues to have no change in her pigmented, posterior lesion; this lesion has now been under observation by various physicians for 15 years without evidence of growth. Several other patients falling into various categories continue to return for intermittent follow-up examinations.

Significance to Biomedical Research and the Program of the Institute: This study has demonstrated that it is possible to differentiate some malignant melanomas from other pigmented ocular tumors. The lack of fine discrimination in this test suggests that improvements of methodology and instrumentation are required before it might have general clinical usefulness. The fact that ^{125}I is a gamma emitter continues to draw attention toward continued use of this, or similar radioisotope-labelled drugs, for diagnosis. Follow-up information concerning ocular tumor patients is being gathered. This can be correlated to their diagnostic work-up.

Proposed Course: It is not appropriate to attempt to improve the methodology or the instrumentation related to this test within the Clinical Branch of the NEI. The results of this study are being prepared for publication. Follow-up of the patients will continue in order to define more clearly their course and the diagnosis of their lesions.

NEI Research Program: Retinal and Choroidal Diseases - Tumors

Experimental Subject or Tissue Source: Human

Research Objective: Diagnosis

Publications:

Gaasterland, D.E.: Systemic radiation during the radioactive phosphorus uptake test. Letter to the Editors. Am. J. Ophthalmol. 81: 691, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00030-06 CB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Studies of Parameters of Intraocular Pressure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB NEI
Others:	Carl Kupfer	M.D.	Director	NEI
	Lessie McCain	R.N.	Clinical Technician	CB NEI
	Helen MacLellan	M.S.	Biologist	CB NEI
	Roy Milton	Ph.D.	Head, Section on Biometry	OBE NEI
	John Pederson	M.D.	Clinical Associate	CB NEI

COOPERATING UNITS (if any)

Normal Volunteer Office, CC, NIH, Pharmaceutical Development Service, NIH, Biomedical and Engineering Instrumentation Branch, DRS, NIH

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.2	0.1	1.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

In this continuing study of the parameters of intraocular pressure, young and old normal volunteers and patients with glaucoma and ocular hypertension participate. There is interest in determining the actual values of the parameters in eyes not affected by medications, and in determining the effects of anti-glaucoma medications alone and in combination upon the parameters in normal and in diseased eyes.

Project Description:

Objectives: To evaluate parameters of intraocular pressure in normal eyes, and eyes with ocular hypertension or glaucoma, before and after anti-glaucoma medications.

Methods Employed: Replicate studies are done upon sophisticated human participants. Seven parameters are determined before and after medication: intraocular pressure, episcleral venous pressure, total facility, true facility of outflow, pseudofacility, aqueous flow, and ocular rigidity. The P_k of Goldmann is no longer determined because it does not add any more information. Acute drug effects are emphasized. Chronic drug effects are studied by use of the Ocusert system (Alza Laboratories) for pilocarpine and in patients receiving monocular treatment in the Ocular Hypertension Protocol of Dr. E. Ballintine (Z01 EY 00150-04-CB).

Major Findings: The major emphasis during this period has been to acquire additional data on acute effects of medications upon the eyes of old normal volunteers and on chronic effects of pilocarpine upon the eyes of ocular hypertension patients. To this end 277 examinations have been scheduled, and 186 examinations of normal volunteers or patients have been carried out in the Glaucoma Research Laboratories during the interval covered by this report. These have provided additional information concerning effects of topical catecholamines, parasympathomimetics, and placebo.

Combined treatment of the eyes of young normal volunteers with topical epinephrine and pilocarpine causes reduced intraocular pressure. The mechanism whereby this occurs is interesting in that either of these medications raises facility of outflow, and together they are additive; but, in contrast, either of these medications alone reduces aqueous inflow and together, the effects are no longer observed. The one medication inhibits the other's effect on aqueous inflow.

Continued development of the fluorophotometer with studies of its accuracy have resulted in creation of a separate protocol for clinical investigation of aqueous flow. This segment of the work has therefore been split off from the present report, and is reported separately (Z01 EY 00050-01-CB).

Significance to Biomedical Research and the Program of the Institute: Study of patterns of alteration of parameters of intraocular pressure by glaucoma medications allows clearer understanding of their mechanisms of action. Studies of parameters more clearly define the difference between normal and abnormal. The measurements can be extrapolated to more basic physiologic functions, yielding insight to the function of the human eye. This information is unique in ophthalmic research.

Proposed Course: The project will be continued.

NEI Research Program: Glaucoma - Etiology of Glaucoma (Primary Glaucoma--Open-Angle Glaucoma/Secondary Glaucomas)

Project No. Z01 EY 00030-06 CB

Experimental Subject or Tissue Source: Human

Research Objective: Etiology, Diagnosis, Treatment

Publications:

Gaasterland, D.E. and Kupfer, C.: Effects of combined treatment with epinephrine and pilocarpine on parameters of intraocular pressure. Presented at the meeting of the Association for Research in Vision and Ophthalmology, Sarasota, Florida, April, 1977.

Kollarits, C.R., Gaasterland, D., Dichiro, G., et al: Visual loss in a patient with orbital varices and ipsilateral glaucoma. Ophthalmic Surg. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00006-06 CB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Research in Methods of Evaluating Visual Processes

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ralpn D. Gunkel	O.D.	Ophthalmic Physicist	CB NEI
Others:	Donald R. Bergsma	M.D.	Senior Staff Ophthalmologist	CB NEI
	Doris Collie		Technician	CB NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION
Psychophysics

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.2	0.8

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The general purpose and intent of this project is to conduct tests, research, and experiments directed toward the use and improvement of clinical procedures for measuring functions or properties relating to vision and the eyes. This includes subjective measurements of visibility and chromaticity thresholds and electrophysiological (objective) measurements as in electroretinography and electro-oculography, and physical measurements such as curvature, hardness, elasticity, and transparency.

The loose definition of this project permits a degree of freedom which has been advantageous to other workers and projects in utilizing certain types of expertise, instruments, and materials.

PHS-6040

Project Description:

Objectives: To discover and utilize the most effective and least traumatic methods for quantitating and evaluating any changes in the eye or its adnexae brought about by disease conditions, toxic materials or degeneration. Objective methods are desired, but are not always attainable. The obvious goal of this project continues to be the maintenance or restoration of normal visual function wherever possible.

Methods Employed: Commercially available instruments and those developed here are used in measuring rod and cone thresholds, color vision, and other ocular functions in clinic patients. There is frequent consultation with Clinical Associates and staff members regarding test methods and results, applicability, interpretation, new ideas, and properties of materials.

Major Findings: Psychophysical tests were done on 420 subjects for the purpose of evaluating or diagnosing toxic, inflammatory, degenerative or congenital retinal conditions.

Various optical, electrical and mechanical devices were designed and/or constructed for use in the projects of other staff members.

The most notable outgrowth of the project has been the confirmation and extension of earlier findings in measuring color vision with the chromagraph. It is now clear that the traditional terms of protanopia, deuteranopia, (red blindness and green blindness) and tritanopia are not properly descriptive of the results obtained with the conventional color tests. Heretofore there have been many cases where the ordinary tests do not distinguish between protanopia and deuteranopia. They never give an accurate measure of the severity of a defect, and they rarely give any definite indication of weakness to colors other than red and green. On the other hand, the chromagraph leaves no doubt as to which colors are seen, which are not seen, and the saturation required for the discrimination of each.

Enough color tests (over 400) have been performed on the chromagraph and compared with the three conventional test methods to establish standards for normal and defective color vision of any type.

Significance to Biomedical Research and the Program of the Institute: Data and information obtained in psychophysical tests contributes materially to the clinical program, and various devices have been of considerable value to other research projects.

Since specific color defects are so easily described with the chromagraph, it seems possible that some of the acquired deficiencies may be correlated with certain ocular pathologies as a diagnostic aid.

Furthermore, since all of the likely types of color defect now appear to be well-established (which could not be done with the conventional tests), it seems inevitable that this system will eventually be adopted for definitive color testing in other eye clinics.

Proposed Course: The project will be continued.

NEI Research Program: Retinal and Choroidal Diseases

Experimental Subject or Tissue Source: Human

Research Objective: Diagnosis, Treatment

Publications:

Gunkel, R.D. and Cogan, D.G.: Colorimetry by a new principle. Arch. Ophthalmol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00018-03 CB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Ophthalmologic Screening for Metastatic Lesions to the Eye

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Muriel Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB NEI
Other:	Joan Bull	M.D.	Senior Staff Fellow	CB NCI

COOPERATING UNITS (if any)

National Cancer Institute

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.36	.26	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the purpose of this project to determine the incidence of metastatic eye disease in patients with metastatic breast carcinoma as well as to evaluate the effects of irradiation on the eye in patients who have metastatic disease and receive irradiation in conjunction with chemotherapy. The response of choroidal lesions to therapy may serve as a measurable lesion for indication of response elsewhere in the body.

Project Description:

Objectives: To determine the incidence of metastatic eye disease in patients with metastatic breast carcinoma, to evaluate the effects of irradiation on ocular tumors which threaten central vision and monitor the effects of irradiation on the eye, and to evaluate the effectiveness of the hormonal manipulations and chemotherapy on ocular metastasis in relation to systemic effect on tumor.

Methods Employed: All NCI patients having metastatic breast carcinoma are examined ophthalmoscopically. Those patients having metastatic disease to the eye are then followed frequently as indicated. The course of the ocular metastatic disease is followed with serial color and infrared fundus photography, Goldmann perimetry and fluorescein fundus photos when indicated.

Major Findings: To date approximately 100 patients have been seen and of those approximately 13 patients have had evidence of ocular metastasis. Several patients have been found to have developed secondary keratitis following radiation therapy to the posterior choroid.

Significance to Biomedical Research and the Program of the Institute: The response of choroidal metastatic lesions to cancer chemotherapy could serve as an indication of response to metastatic disease elsewhere in the body.

Proposed Course: To continue for one additional year.

NEI Research Program: Retinal and Choroidal Diseases - Tumors

Experimental Subject or Tissue Source: Human

Research Objective: Diagnosis, Treatment

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 EY 00011-03 CB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Pigment Dispersion With and Without Glaucoma

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Muriel Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB NEI
Other:	Luis DelValle	M.D.	Staff Fellow	CB NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.90	.70	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The purpose of this project is to compare patients having pigment dispersion syndrome with and without glaucoma. The acquired data may enable a determination of the risk of patients with pigment dispersion syndrome to develop glaucoma as well as to understand more of the pathology of the disease state.

PHS-6040

Project Description:

Objectives: To compare patients having pigment dispersion with and without glaucoma by documenting and following the clinical features and course of their disease, and by evaluating the patient's performance on a variety of diagnostic tests. To determine the presence of abnormal aqueous humor dynamics using provocative testing in those patients having pigmentary dispersion with and without glaucoma. To compare pigment dispersion with and without glaucoma with respect to possible genetic markers (i.e. lymphocyte transformation, HLA and ABO antigens and family history of open-angle glaucoma). To determine whether pupillary dynamics to light stimulation are abnormal in cases having iris transillumination.

Methods Employed: At the first visit, the following examinations are performed:

Complete family history with detailed pedigree
Best corrected visual acuity with manifest refraction
Slit lamp examination
Visual field examination (Goldmann I_{2e} and I_{4e})
Applanation Goldmann tension (app)
Photography of iris transillumination
Goniophotography
Blood specimen for HLA and ABO antigen typing

At the next visit, the following examinations are performed:

Static perimetry
Base-line tonography and water-drinking tonography one hour later
Fasting blood sugar when indicated

At the third visit, the following examinations are performed:

Slit lamp photography of Krukenberg spindle
Dilated ophthalmoscopic examination (10% phenylephrine and 1% cyclogel)
Stereophotographs of the optic nervehead

At the fourth visit, pupillography is performed.

Major Findings: Patients may have pigment dispersion syndrome for as long as 20 years without developing glaucoma.

There may be a hereditary predisposition in some cases, as seen in a mother and daughter, two brothers, and a brother and sister.

The steroid testing and PTC taste testing do not appear to show any particular categorization of these patients. Recent evidence has indicated that HLA antigens are also not significantly different than the normal population.

It may be noted that whether filtering procedures are performed or not, pigment may be lost from the trabecular meshwork in time.

Significance to Biomedical Research and the Program of the Institute: These data may enable a determination to be made of the risk of patients having pigment dispersion to develop glaucoma. It may be possible to identify which features of these determinations may have predictive value in forecasting those patients having pigment dispersion who may develop a field defect. In addition, the relationship of "pigmentary" glaucoma to the known characteristics of open-angle glaucoma can be investigated.

Proposed Course: This project will be continued for four more years

NEI Research Program: Glaucoma - Etiology of Glaucoma (Developmental Glaucomas/Secondary Glaucomas)

Experimental Subject or Tissue Source: Human

Research Objective: Diagnosis, Treatment

Publications:

Kaiser-Kupfer, M.I. and Mittal, K.K.: The HLA and ABO antigens in pigment dispersion syndrome. Am. J. Ophthalmol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00042-01 CB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Progressive Essential Iris Atrophy

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Muriel I. Kaiser-Kupfer	M.D.	Senior Staff Ophthalmologist	CB NEI
Other:	Carl Kupfer	M.D.	Director	NEI
	Rodney Lynk	M.D.	Medical Officer	CB NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.56	.44	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients are being recruited with progressive essential iris atrophy associated with or without corneal disease. Information is being gathered to evaluate the clinical features and course of the disease process, to investigate aqueous humor dynamics in both affected and unaffected eyes and to attempt to find genetic markers such as HLA and ABO antigens or physical correlates with the disease process.

Project Description:

Objectives: The objectives of the study are to develop a panel of patients with progressive essential iris atrophy and to study these patients in depth to determine factors which may aid in understanding the pathophysiology of the disease process and to study the natural history of this disease. Measurements of aqueous humor dynamics, genetic markers such as HLA and ABO antigens, physical correlates and iris fluorescein angiography to determine the role of the vasculature will be carried out.

Methods Employed: During the course of the evaluation the following procedures are performed:

Complete family history with detailed pedigree
Best corrected visual acuity with manifest refraction
Slit lamp examination
Visual field examination (Goldmann I_{2e} and I_{4e})
Photography of iris and iris transillumination
Gonioscopy and goniophotography
Iris fluorescein angiography and photography
Baseline tonography
A complete medical and dental evaluation
Dilated ophthalmoscopic examination
Stereophotographs of the optic nervehead

Major Findings: Histopathologic and electron microscopic study of iris tissue and trabecular meshwork tissue has not indicated any clues to the pathogenesis of the disease process.

An ultrathin corneal contact lens is useful in certain patients to prevent recurrent rupture of corneal bullae.

Significance to Biomedical Research and the Program of the Institute: These data may contribute to an understanding of pathophysiologic factors involved in the rare entity of progressive essential iris atrophy. In addition, a careful study of the progression of the disease from the earliest signs will clarify the significance of corneal involvement and the status of outflow channels which may add to the understanding of the mechanism of glaucoma.

Proposed Course: The project will continue for four more years.

NEI Research Program: Glaucoma - Etiology of Glaucoma (Developmental Glaucomas/Secondary Glaucomas)

Experimental Subject or Tissue Source: Human

Research Objective: Diagnosis, Treatment

Publications:

Kaiser-Kupfer, M., Kuwabara, T., and Kupfer, C.: Progressive bilateral essential iris atrophy. Am. J. Ophthalmol. 83: 340, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00040-01 CB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Visual Function and Ocular Pigmentation in Albinism

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Muriel I. Kaiser-Kupfer M.D. Senior Staff Ophthalmologist CB NEI
Other: None

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
.20	.15	

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Patients with hypomelanotic disorders such as ocular albinism, oculo-cutaneous albinism, Chediak-Higashi Disease, Hermansky Pudlak Syndrome and Iris transillumination defects are being recruited as well as family members to determine visual function and to evaluate the course of visual function with time. Family members are evaluated to attempt to determine factors which may identify the heterozygous state.

Project Description:

Objectives: The objectives of the study are to relate the level of visual function to the amount of ocular pigmentation, especially iris and retinal pigmentation; to correlate the amount of nystagmus with visual acuity and iris pigmentation; to determine whether ocular pigmentation, visual acuity and nystagmus change with age; and to identify the heterozygous state in family members.

Methods Employed: The following examinations are performed:

- Complete family history with detailed pedigree
- Best corrected visual acuity at near and distance with refraction
- Slit-lamp examination
- Nystagmus recording using eye movement monitoring EOG
- Psychophysical testing including D-15 and Munsell 100 hue, rod and cone thresholds
- Dilated ophthalmoscopic examination
- Hair bulb incubation
- Photography to document hair color, eye color, skin color, iris transillumination, disc and macula

Examination of family members includes:

- Best corrected visual acuity
- Slit-lamp examination of iris
- Photography of iris transillumination
- Fundus examination when vision not corrected to 20/20

Major Findings: Examination of patients and family members indicates that the finding of transillumination of the iris may be seen in the absence of recognized albinism. The pattern appears to be punctate and may be present in a diffuse manner or limited to the 6 o'clock sector.

Significance to Biomedical Research and the Program of the Institute: These data may allow identification of the carrier state in albinism which would be of importance in genetic counselling. In addition, it may be possible to determine whether the development of the fovea is abnormal in albinism and the cause of the decreased visual acuity or whether the decreased visual acuity is secondary to the hypopigmentation and light-scatter and glare that results. In addition, it will be possible to ascertain whether there is an improvement of the visual acuity with age and correlated with changes in pigmentation.

Proposed Course: This project will be continued for five more years.

NEI Research Program: Retinal and Choroidal Diseases - Developmental and Hereditary Disorders

Project No. Z01 EY 00040-01 CB

Experimental Subject or Tissue Source: Human

Research Objective: Diagnosis, Treatment

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00013-06 CB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Study of Pharmacodynamics of Various Agents Affecting the Intraocular Pressure

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Frank J. Macri	Ph.D.	Pharmacologist	CB NEI
Other:	None			

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.0	1.0	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Clonidine, in the enucleated arterially perfused cat eye, was found to produce a decrease in the rate of aqueous humor production. The mechanism of action for this response has been ascribed to a vasoconstriction of afferent ciliary process blood vessels to decrease ultrafiltration.

Cold, as a stimulus to the cornea, was found to produce an easily reversible decrease in aqueous humor production. The mode of action of this response is being studied.

Changes of intraocular pressure, arterial pressure or of osmotic pressure induced no local intraocular reflexes which influenced aqueous humor production.

Project Description:

Objectives: To determine the pharmacodynamics of agents able to alter the intraocular pressure (IOP) with a view to finding more effective compounds and possibly to furthering the understanding of mechanisms which maintain the intraocular pressure.

Methods Employed: Studies are made on the enucleated, arterially perfused cat eye. Perfusate is channeled through the ophthalmic artery to nourish the entire eye, or a ligature is placed around the optic nerve at its insertion, so that only the anterior segment of the eye is perfused. Drugs and other test substances are added to individual bottles of perfusate fluid which can then be introduced into the system by stopcock control. Temperature and rate of arterial flow are easily regulated. The rate of aqueous humor formation was estimated by determining the rate of decay of intracamerally injected I^{125} tagged serum albumin.

Major Findings:

Clonidine

Clonidine (Catapres), a well-known antihypertensive agent, has been used, primarily in Europe, to decrease intraocular pressure. Its mechanism of action on the eye was uncertain since it always produced a parallel reduction of systemic blood pressure. Utilizing our enucleated, arterially perfused cat eye preparation, we have demonstrated that Clonidine produces a vasoconstriction in the anterior segment of the eye by direct stimulation of α-adrenergic receptors. Clonidine also produces a fall in the rate of aqueous humor production. These two findings suggest that Clonidine acts much like phenylephrine and dopamine to reduce eye pressure.

Local reflexes which can offset intraocular pressure

We have made a search for local reflexes of the eye which could possibly alter IOP. In the course of these studies, we have found no reflex actions on either IOP or aqueous humor inflow rates. The stimuli applied to the eye were changes of intraocular blood pressure, intraocular pressure or changes of arterial perfusate osmotic pressure. We have found, however, a significant decrease in the rate of aqueous humor production and IOP when cold is applied to the cornea. The response is not mediated by neurogenic corneal receptors since xylocaine could not abolish the response. The response also is not mediated through the intraocular E-1 receptors. The mechanism of action for the ocular effects still remains to be elucidated.

Central neuronal connection of E-1 intraocular receptors

Although we have demonstrated nicotinic receptors in the eye, which when stimulated lower IOP and aqueous flow, the physiologic significance of these receptors is unknown. If we can demonstrate a connection of these

receptors to extraocular nerves, central control would then be implied and a possible physiologic role indicated. Stimulation of extraocular sympathetic nerves does not activate these receptors. Currently, studies are under way to determine if these receptors are under p-sympathetic control.

Significance to Biomedical Research and the Program of the Institute: The sympathomimetic nature of Clonidine action on the vasculature of the eye and on aqueous humor formation mimics the responses reported last year for phenylephrine, hydroxyamphetamine, and dopamine. This lends additional support to our concept that vasoconstriction of afferent ciliary process blood vessels decrease aqueous humor formation. These findings also demonstrate that the clinical application of Clonidine to the eye can decrease IOP by decreasing the inflow rate of aqueous humor in addition to its centrally mediated responses. Admonition by certain individuals that Clonidine should not be used clinically on the eye because of its vasoconstrictive actions appears open to question. The vasoconstrictive action of Clonidine is identical to that produced by phenylephrine and hydroxyamphetamine, drugs which have been in general ophthalmic use for many years.

The finding of an apparent reflex decrease of aqueous humor production and IOP resulting from cold application to the cornea was unexpected. The physiologic significance and mechanism of action of this phenomenon remains to be determined.

Although there are reports to indicate regulation of intraocular pressure through the sympathetic nervous system, we have not been able to demonstrate any sustained effect of sympathetic nerve stimulation on either IOP or aqueous humor formation. This is important since we had previously anticipated that the E-1 receptors of the eye (those which constrict afferent ciliary process blood vessels) were part of this system. Present evidence is accumulating, however, to indicate that the E-1 receptors are under p-sympathetic control as are the E-2 receptors (those which constrict efferent ciliary process blood vessels).

Proposed Course: We anticipate continuing to study the mechanism of the cold response of the cornea. This may uncover pathways (either neurogenic or chemical) which could, by a local response, affect the IOP. We also intend to continue studies to determine if the E-1 receptors of the eye are in communication with the central nervous system. If they can be found to be so, the implication would be that these receptors are under control of the brain, and thus their action can be centrally regulated.

Recent reports indicate that Timolol, a B-adenergic blocking agent, is effective in lowering the IOP in glaucoma patients. The mechanism for this response is unclear. We intend to study this compound in the enucleated, arterially perfused cat eye in order to determine how the IOP is lowered and to elucidate the pharmacologic mechanism of the response.

NEI Research Program: Glaucoma - Hydrodynamics of the Eye

Project No. Z01 EY 000013-06 CB

Experimental Subject or Tissue Source: Cat

Research Objective: Etiology

Publications:

Macri, F.J. and Cevario, S.J.: Blockade of the ocular effects of acetazolamide by phencyclidine. Exp. Eye Res. 24: 121-127, 1977.

Macri, F.J. and Cevario, S.J.: The inhibitory actions of dopamine, hydroxyamphetamine and phenylephrine on aqueous humor formation. Exp. Eye Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE **NOTICE OF INTRAMURAL RESEARCH PROJECT**	PROJECT NUMBER Z01 EY 00052-01 CB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cell and Tissue Interactions in the Production of Corneal Collagenase

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David A. Newsome	M.D.	Investigator	CB NEI
Other:	Jerome Gross	M.D.	Massachusetts General Hospital	

COOPERATING UNITS (if any)
Developmental Biology Laboratory,
Massachusetts General Hospital and Harvard Department of Medicine
Boston, Massachusetts

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This study of alkali-burned rabbit corneas was designed to understand more about the cellular and tissue regulatory responses that control the elaboration of the enzmye collagenase, one of the chief factors responsible for corneal ulceration and melting. The present project is to 1) document and localize the elaboration of collagenase by corneal tissues, 2) examine the interaction of blood monocytes and their products with corneal cells in stimulating enzyme production, 3) examine the interaction of regenerated and normal corneal epithelium and stroma in enzyme production, and 4) learn more about the regulation of collagenase production and collagen synthesis by corticosteroid and progestational hormones.

PHS-6040

Project Description:

Objectives: The goal of this project is to understand mechanisms of stimulation and inhibition of collagenase production in corneal tissue, since this enzyme plays an important role in corneal melting and ulceration associated with various pathological conditions.

Methods Employed: Normal corneas and alkali-burned corneas from young adult albino rabbits provided explants and sources of cell populations for cell cultures. Blood monocytes were prepared by centrifugation on a Ficoll-Hypaque gradient. In the hormone studies, radioactive proline incorporation in total protein and as hydroxyproline in collagen was determined using stream splitting on the amino acid analyzer. Collagenase was measured using ^{14}C-labeled collagen gel technique.

Major Findings:

Tissue source of corneal collagenase

Stroma cells which migrated out of explants of alkali-burned tissue produced the greatest amounts of collagenase. This enzyme production persisted through about two serial passages, after which it became undetectable. In a few cases, isolated epithelium from alkali-burned corneal explants was associated with transient, low-level enzyme production. In normal corneal tissue, small amounts of enzyme production were observed only with early cultures of stromal cells.

Stimulation of collagenase production by blood monocytes

The addition of peripheral blood monocytes, or medium in which these monocytes had been incubated, to cultures of corneal stromal cells resulted in a stimulation of collagenase production. Monocytes from rabbits with alkali-burned corneas produced about a two-fold greater effect than did cells from normal animals.

Corneal epithelial-mesenchymal interactions

Cultures of alkali-burned stromal cells that had ceased to produce detectable collagenase were stimulated to produce significant amounts of enzyme when added to cultures of epithelium from similar corneas. Normal epithelium also stimulated enzyme production in alkali-burned stromal cells but not in normal stromal cells. The medium from epithelial cells was also stimulatory.

Hormonal effects on collagenase production and collagen synthesis

Both the corticosteroid dexamethasone and the semi-synthetic hormone medroxyprogesterone decreased collagenase synthesis as well as total protein synthesis in vitro. The effect of dexamethasone was more marked than that of medroxyprogesterone, and the depression of collagen synthesis preferentially greater. Basement membrane collagen production, as judged by synthesis of

3-hydroxyproline, was also more depressed by dexamethasone.

Significance to Biomedical Research and the Program of the Institute: These investigations have added to knowledge of the cellular and tissue regulatory mechanisms involved in the elaboration of the tissue-destructive enzyme collagenase. Control of such enzymes is useful in treating a variety of serious corneal diseases.

Proposed Course: The mechanism and site of hormonal influence on collagenase production in alkali-burned corneas will be investigated further.

NEI Research Program: Corneal Disease - Corneal Transplantation and Stromal Injury and Repair

Experimental Subject or Tissue Source: Rabbit

Research Objective: Etiology

Publications:

Newsome, D.A. and Gross, J.: Cellular regulation of corneal collagenase: Stimulation of serially passaged stromal cells by blood mononuclear cells. J. Exp. Med. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00050-01 CB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Aqueous Humor Flow Measurement by Fluorophotometry

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jonathan E. Pederson	M.D.	Clinical Associate	CB NEI
Other:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB NEI
	Helen M. MacLellan	M.S.	Biologist	CB NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.4	0.3	0.1

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This work attempts to measure the rate of aqueous humor flow in humans by determining the rate of loss of fluorescein from the eye after iontophoresis of fluorescein into the cornea. The aqueous humor flow is also calculated from tonographic results in the same individuals, allowing a comparison of the two methods. Individuals with normal, low, or high intraocular pressure are studied to examine the effect of intraocular pressure on aqueous flow.

The accuracy of the aqueous flow rate measured by fluorophotometry was evaluated in freshly enucleated monkey eyes. The calculated flow rate averaged 5% lower than the known perfusion rate.

PHS-6040

Project No: Z01 EY 00050-01 CB

Project Description:

Objectives: This project is designed to measure directly aqueous humor flow in humans with a fluorophotometer. The first objective is to compare the results with a method of indirect calculation of flow from tonography. The second objective is to determine the relationship between intraocular pressure and aqueous humor flow.

Methods Employed: A cylindrical piece of polyacrilamide gel is saturated with fluorescein solution. The gel is touched to the cornea, and fluorescein is deposited due to a small current provided by a dry cell battery. A photomultiplier tube with appropriate filters, mounted on a slitlamp biomicroscope, measures the total amount of fluorescein in the eye, as well as the aqueous concentration. Illumination is provided by a chopped light source. The photomultiplier tube signal is fed to a tuned amplifier. The rate of loss of fluorescein from the eye as a function of time yields the flow rate of aqueous humor.

Major Findings: The accuracy of the aqueous flow rate measured by fluorophotometry was evaluated in freshly enucleated monkey eyes. The calculated flow rate averaged 5% lower than the known perfusion rate. This establishes the procedure as a useful tool in the measurement of aqueous flow. Preliminary studies have been performed in a few individuals and yielded reproducible results. The aqueous flow measured by fluorophotometry tends to exceed the flow calculated from tonography.

Significance to Biomedical Research and the Program of the Institute: The aqueous humor flow rate is a primary determinant of the intraocular pressure. An accurate, safe, and reproducible determination of the flow rate in humans under normal and pathological conditions will lead to increased understanding of glaucoma and hypotony.

Proposed Course: The human studies will continue. After an examination of symmetry between paired eyes of the same individual, drug studies will be initiated, using those agents commonly employed in the treatment of glaucoma.

NEI Research Program: Glaucoma

Experimental Subject or Tissue Source: Human/Rhesus monkey

Research Objective: Etiology

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00046-01 CB

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Laboratory Studies of Aqueous Humor Dynamics

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jonathan E. Pederson	M.D.	Clinical Associate	CB NEI
Other:	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB NEI
	Helen M. MacLellan	M.S.	Biologist	CB NEI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Clinical Branch

SECTION

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.6	0.4

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Several interrelated projects to investigate intraocular fluid movement in rhesus monkeys have been initiated. 1) Intraocular vascular reabsorption of aqueous humor was found to be 10% of the total amount of aqueous humor leaving the eye. This reabsorption occurs by a pressure-dependent flow into uveal vessels. 2) The amount of reflux of fluid across the trabecular meshwork from Schlemm's canal during hypotony was studied. The amount of reflux was 7% of the aqueous humor production rate. This establishes that the outflow pathway is a virtual one-way valve. 3) A comparison was made of the change in outflow facility during constant pressure perfusion with pooled aqueous humor versus glutathione-bicarbonate Ringer's solution. Ringer's solution caused a progressive increase in outflow facility, whereas aqueous humor did not. 4) The composition of aqueous humor from pooled samples has been under study. 5) An unsuccessful attempt was made to determine the intraocular pressure (P_k) at which aqueous formation ceased by photographing the appearance of fluorescein on the ciliary process after intravenous injection. 6) A study has been started of the effect on intraocular pressure of injecting various solutions into the suprachoroidal space.

Project Description:

Objectives: This project is designed to examine the physiology of intraocular fluid movement under varied experimental conditions. The major emphasis is on conventional and secondary outflow mechanisms.

Methods Employed: Various methods of perfusion and cannulation of the eye with subsequent measurement of pressures, flows, and concentrations of various substances were performed.

Major Findings:

Uveal reabsorption of aqueous humor

The concentration of fluorescein and radioiodinated serum albumin were measured in the vortex vein of the rhesus monkey during anterior chamber perfusion of these substances at two different intraocular pressures. Only a tiny amount of albumin appeared in the vortex vein blood, but a rapid excess of fluorescein above that in the plasma appeared. This excess was pressure-dependent, suggesting an ultrafiltrative uptake into uveal vessels.

Reflux fluid movement across Schlemm's canal during hypotony

The anterior and posterior chamber concentrations of labeled sucrose and inulin were measured during constant intravenous infusion of these substances. At an intraocular pressure of 2 mmHg, amounts of sucrose and inulin equivalent to 7% of the aqueous volume produced by the eye refluxed back into the anterior chamber. The resistance of reverse fluid movement was calculated to be about 50 times greater than the resistance to fluid movement in the normal direction.

Perfusates and the washout phenomenon

The facility of outflow was measured by constant pressure perfusion in monkey eyes. Using pooled monkey aqueous humor and glutathione-bicarbonate Ringer's solution as perfusate. Pooled aqueous humor did not cause a change in facility, but Ringer's solution caused a progressive increase in facility. This was not due to pH or change in ascorbate concentrations.

Chemical composition of aqueous humor

A systematic analysis of the chemical composition of monkey aqueous humor was begun in order to create an ideal perfusing solution for experimental studies of aqueous humor dynamics.

Fluorescein cycloscopy

Iridectomized monkeys were examined gonioscopically during intravenous infusion of fluorescein. An attempt was made to determine the intraocular pressure at which fluorescein would not appear on the ciliary processes. The rapid diffusion of fluorescein into the posterior chamber, even at high intraocular pressures, precluded an accurate determination.

Hypotony and choroidal detachment

A study of the effect of choroidal detachment on intraocular pressure was initiated. A comparison between solutions of varying composition injected into the suprachoroidal space is under investigation. Preliminary results suggest a central role of reduced protein movement out of the eye as a causative mechanism for the hypotony of choroidal detachment.

Significance to Biomedical Research and the Program of the Institute: These studies should elucidate the normal dynamics of aqueous humor as well as the abnormal dynamics in experimentally induced situations, mimicking clinical problems. Ultimately, these studies may yield information applicable to glaucoma and hypotony.

Proposed Course: Similar studies will be continued. Particular emphasis will be placed on the perfusate effects on outflow resistance, and the interrelationship between choroidal detachment and intraocular pressure.

NEI Research Program: Glaucoma - Hydrodynamics of the Eye

Experimental Subject or Tissue Source: Rhesus monkey

Research Objective: Etiology

Publications:

Pederson, J.E., Gaasterland, D.E., and MacLellan, H.M.: Uveoscleral aqueous outflow in the rhesus monkey: Importance of uveal reabsorption, Invest. Ophthalmol., in press, 1977.

Gaasterland, D.E., Pederson, J.E., and MacLellan, H.M.: Perfusates and the "Washout Phenomenon", presented at the Association for Research in Vision and Ophthalmology Meeting, Sarasota, Florida, April 1977.

Green, K., Sherman, S.H., Laties, A.M., Pederson, J.E., Gaasterland, D.E., and MacLellan, H.M.: The fate of anterior chamber tracers in the living rhesus monkey eye with evidence for uveovortex outflow. In Cant, J.S. (ed.): Intraocular Fluid Dynamics. Oxford, Oxford University Press (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00056-01 CB

PERIOD COVERED

July 1, 1976 To September 30, 1977

TITLE OF PROJECT (80 characters or less)

Transport Mechanisms in the Ciliary Epithelium

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Richard A. Stone	M.D.	Clinical Associate	CB NEI
Other:	Elmer J. Ballintine	M.D.	Clinical Director	CB NEI
	Richard Weiblinger	B.S.	Biologist	CB NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.3	0.2	0.1

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The transepithelial transport mechanisms of isolated ciliary body and iris preparations obtained from normal rhesus monkey eyes are being studied. Concentration of para-aminohippurate by these preparations has been demonstrated. Tissue culture techniques for the iris and ciliary body pigmented epithelium are being developed in anticipation of exploring anion transport mechanisms in cells grown in culture.

PHS-6040

Project Description:

Objectives: Several mechanisms for the uptake of organic and inorganic anions have been described in rabbit eyes. There is some published evidence indicating that these processes also exist in primate eyes, but the uptake of organic and inorganic anions by primate iris and ciliary body has not been systematically explored in vitro.

The main objective of this project is to expand our understanding of these transport systems as they occur in the primate eye.

Methods Employed: Specimens of iris and ciliary body are removed from the eyes of rhesus monkeys when they become available from animals used by the FDA Bureau of Biologics in their tests of vaccines. These tissue specimens are then incubated with various radio-labeled anions and inhibitors of transport. The rate of entrance of the anion into the tissue and the tissue/medium are measured. We are attempting to develop a technique for obtaining relatively pure cultures of iris and ciliary pigment epithelium which can be used for similar studies of anion transport.

Major Findings: The anion transport systems studied in the monkey to date appear to have the same general characteristics as the systems described in the rabbit, although they are somewhat less active.

Significance to Biomedical Research and the Program of the Institute: There are important unsettled questions regarding the mechanism of aqueous secretion in the control of the aqueous composition. Much of our knowledge is based on research in nonprimate systems, and the results are not always immediately applicable to man. This project is aimed at increasing our knowledge of the mechanism of aqueous formation and control in the primate eyes. This knowledge is a basis for predicting what chemical agents might be useful in inhibiting aqueous humor formation for the treatment of glaucoma.

Proposed Course: The project will be continued as outlined above.

NEI Research Program: Glaucoma - Hydrodynamics of the Eye

Experimental Subject or Tissue Source: Monkey

Research Objective: Etiology

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00044-01 CB

PERIOD COVERED

December 12, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Organ Culture of the Normal and Dystrophic (RCS) Rat Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Makoto Tamai M.D. Visiting Scientist CB NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Study of the phagocytic function of pigment epithelium in RCS dystrophic rats, thought to be important in the process of photoreceptor degeneration, was undertaken with the organ culture technique. The pigment epithelium of the RCS rat was cultured with the normal neural retina or vice versa and examined with the light and electron microscopy.

PHS-6040

Project No. Z01 EY 00044-01 CB

Project Description:

Objectives: One of the important biological functions of the pigment epithelium is the uptake of shed photoreceptor outer segments. If this activity is disturbed, accumulation of the outer segment debris occurs and may lead to visual cell death. These processes have been accepted as the cause of retinal dystrophy in RCS rats, but no one knows if the defect exists in the pigment epithelium, in the outer segments, or in both. These problems were studied in normal and dystrophic (RCS) rats in vitro.

Methods Employed: Techniques for organ culture of the developing rat retina and pigment epithelium were used. Normal neural retina of the post-natal seven to twelve day, as well as the dystrophic ones of the same age, was cultured with RCS rat pigment epithelium. Their interactions and the existence of phagosomes were evaluated by light and electron microscopy.

Major Findings: Pigment epithelium of the dystrophic RCS rats phagocytized neither the outer segments from RCS neural retina nor those of the normal strain during the incubation period of up to six days. Pigment epithelium of the normal rats, however, could phagocytise both of them.

Significance to Biomedical Research and the Program of the Institute: The present studies strongly suggest that the membrane characteristics or shedding mechanisms of the outer segments in the dystrophic rats are normal, but their recognition by or systems for their uptake in the pigment epithelium are defective.

Proposed Course: Using the organ culture technique, these experiments will be continued not only in the rat but also in other dystrophic animals.

NEI Research Program: Retinal and Choroidal Diseases - Visual Cells and Pigment Epithelium

Experimental Subject or Tissue Source: Rat

Research Objective: Etiology

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00048-01 CB

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Endothelial Wound Healing of the Cornea

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Teruo Tanishima	M.D.	Visiting Scientist	CB NEI
Other:	Toichiro Kuwabara	M.D.	Head, Section on Experimental Pathology	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Clinical Branch

SECTION

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	0.8	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The healing mechanism of a small wound which had been made on the posterior surface of the rabbit cornea was studied by electron microscopy. Due to the curling characteristic of the cut edge of Descemet's membrane, a tissue defect measuring about 200 μm in width was formed. Endothelial cells in the adjacent area of the wound rapidly slid over the curled Descemet's membrane and then filled up the tissue defect. The cells facing the anterior chamber became the covering endothelium, but many cells in the wound defect transformed into fibroblast-like cells and eventually decreased in number.

Project Description:

Objectives: The wound healing process of the avascular corneal tissue is unique. Early cellular reaction of the endothelial cells to the wound has not been clearly understood.

Methods Employed: Small wounds of the posterior surface of the cornea were made by diagonal insertion of a thin flat needle into the central cornea of albino rabbits. The wounds were examined by electron microscopy at various time intervals. Also, the sliding activity of the endothelium in vitro was examined by electron microscopy. Various metabolic inhibitors were added to the incubation media.

Major Findings: The cut edges of Descemet's membrane curled toward the anterior chamber, and a tissue gap measuring about 200 μm was formed immediately following the wounding. The endothelial cells in the vicinity of the wound began to slide along Descemet's membrane and reached the cut edge three hours after the wounding. The sliding cells contained numerous microtubules in the extending processes. The sliding continued until the tissue defect was filled with the endothelial cells by the 24th hour. The cells facing the anterior chamber became the covering endothelium by forming conspicuous apicolateral junctions and basal lamina whereas the cells piled in the tissue defect began to show a fibroblast-like appearance, losing junctions. These cells produced abundant basal lamina-collagen substances among them and gradually disappeared. The sliding and transformation of the endothelium occurred without mitotic activity. Proliferation of keratocytes was not involved in this early wound healing.

Active sliding of the endothelial cells occurred in an organ culture system. Small pieces of the corneal tissue, the epithelium of which had been removed, was incubated in a tissue culture medium, and the endothelial cells were examined by electron microscopy at various time intervals. Sliding was inhibited by a cold temperature (4^{0}C) of the media and presence of para-hydroxybenzoate (10^{-5}M), iodoacetate (10^{-5}M) and sodium fluoride (10^{-5}M).

Significance to Biomedical Research and the Program of the Institute: For successful corneal transplantation in patients, maintenance of the healthy endothelium of the graft and host is one of the most important factors. Clarification of the role of the endothelium in wound healing is the first step of the related investigation.

Proposed Course: This research will be continued at the Department of Ophthalmology of Tokyo University upon the principal investigator's return.

NEI Research Program: Corneal Diseases - Corneal Transplantation and Stromal Injury and Repair.

Experimental Subject or Tissue Source: Rabbit

Research Objective: Etiology, Diagnosis, and Treatment

Publications: None

Laboratory of Vision Research

ANNUAL REPORT
NATIONAL EYE INSTITUTE
July 1, 1976 - September 30, 1977

REPORT OF THE CHIEF, LABORATORY OF VISION RESEARCH

Jin H. Kinoshita, Ph.D.

At the time of the new fiscal year one conspicuous change is the addition of 2000 sq. ft. of new laboratory space in Building 6. This allows for a modest expansion in the number of investigators. The main addition is Dr. Igal Gery, an accomplished immunologist, who will initiate research activities on ocular immunology. This is an area in the vision research field where a number of good research opportunities have been left unattended because of a lack of sufficient numbers of competent investigators. Dr. Gery, with knowledge of sophisticated concepts and techniques in immunology, brings considerable expertise to the vision research field. The development of an active research program in ocular immunology will not only be an asset to the NEI but to the overall community of vision researchers as well.

As mentioned in previous Annual Reports, a particularly successful continuing activity of the Laboratory of Vision Research is the NEI sponsored symposia held on the Bethesda campus. In October 1976 a Symposium on Experimental Eye Pathology was held and chaired by Dr. T. Kuwabara. Approximately 150 investigators from throughout the country participated in this 3-day meeting, and its proceedings were published in Investigative Ophthalmology and Visual Science. This meeting was acclaimed as one of the best of its kind, and because of its success many have requested that a symposium on this subject be held on a more regular basis.

During the next fiscal year Dr. A.J. Coulombre will organize a symposium sponsored by the National Eye Institute on "Corneal Development" to be held on October 20 and 21, 1977. Among the topics to be considered are: embryological origins of corneal cells, cell population dynamics, development of cell junctions, origins and functions of basement membranes, and clinically recognized anomalies of corneal development. It is the purpose of the symposium to define present positions and near-term opportunities in research on the developing cornea.

Another type of initiative developed by the intramural program of the NEI is demonstrated in the establishment of the National Cooperative Cataract Research Group. The formation of this group was initiated by the cataract section of the LVR. It required the enthusiastic cooperation of 20 participating laboratories involving over 100 investigators who agreed to coordinate their efforts so that progress in research on the human lens and cataracts could be accelerated. Each laboratory has been assigned to carry out specific areas of research. The program will be monitored by an executive committee, while the collection, sorting, and redistribution of data will be done by members of the LVR.

In initiating this program the details of assembling the necessary information into a formal proposal submitted to the National Advisory Eye Council was worked out with the advice and help of the NEI extramural staff. To develop this initiative from outside the intramural program would have been considerably more difficult. If the endeavor is successful, this kind of cooperative team effort focusing on a particular research problem may serve as a model for attacking other clinical problems.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00003-05 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cataracts

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jin H. Kinoshita	PhD.	Chief	LVR NEI
Other:	Shambhu Varma	Ph.D.	Visiting Scientist	LVR NEI
	Peter Kador	Ph.D.	Staff Fellow	LVR NEI
	Lorenzo O. Merola		Gen. Physical Scientist	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	2.5	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It has been shown for the first time that the development of cataracts in an experimental diabetic animal can be delayed by chemical treatment. An inhibitor of aldose reductase, quercitrin, has been shown to effectively delay the onset of diabetic cataracts in an experimental animal.

PHS-6040

Project Description:

Objectives: To study the mechanism of formation of cataracts in experimental animals and to explore possible means by which these cataracts can be prevented.

Methods Employed: Sugar cataracts can be induced in experimental animals by making them diabetic with appropriate chemical agents, or by making them galactosemic or xylosemic with a diet enriched with galactose or xylose. Another approach to studying cataracts is to employ animal models. We have developed a colony of a Nakano mouse strain with hereditary cataracts.

Major Findings: An understanding of the nature of diabetic cataracts evolved from the detailed study of galactose cataracts. These two forms of cataracts fall into a class called sugar cataracts. It is now generally accepted that the common mechanism initiating these cataracts involves aldose reductase. Polyols increase in the lens during cataract formation of diabetic, galactosemic and xylosemic rats. Thus far, experiments do not show that polyols are involved in the inhibition of any key enzymes or that they directly affect vital processes. If this is the case, then the question is how do polyols trigger the events that lead to cataract formation? The answer to this question comes from piecing together certain facts. One fact is that polyols do not penetrate biological membranes very readily so that if polyols are formed within the lens they can accumulate to high levels. This fact led to the idea that in these sugar cataracts the polyols may have an osmotic effect. Support for this possibility comes from histopathological studies. Investigators earlier had made histopathological studies claiming that the earliest histological change to occur is the appearance of hydropic lens fibers. They found that these lens fibers are swollen and the accumulation of fluid is primarily within the lens fibers and not extracellular. Recently, the early swelling of the lens fibers in galactose cataract has been confirmed by electron microscopy. Thus, the morphological studies seem to support the idea that polyols may cause an osmotic change.

The suggestion that polyols may be causing an osmotic effect in the early phase of sugar cataract formation was supported by biochemical studies. In the experiments in which the galactose-fed rats were sacrificed during various periods of time and the lenses analyzed, it was shown that the accumulation of polyol was paralleled by an increase in lens hydration. A better quantitative relationship was demonstrated in organ culture where one lens was incubated in high galactose medium while the contralateral lens was kept in a normal medium. An increase in lens hydration paralleled the increase in polyol accumulation in the galactose-exposed lens. This same relationship was also shown when the lens was exposed to high glucose medium simulating hyperglycemic conditions. In the lens incubated in 35 mM glucose, there was a sudden rise in sorbitol which was accompanied by an increase in lens hydration. The increase in sodium ions was a later phenomenon. These results strongly suggested that in the early stages of the cataract polyol accumulation and not the increase in electrolytes was responsible for the lens swelling.

The most convincing evidence for the polyol-osmotic hypothesis came from in vivo experiments using inhibitors of aldose reductase. In galactosemic rats, systemic administration of an aldose reductase inhibitor effectively delayed the onset of cataract formation. Validation of the hypothesis from experiments with the diabetic animals was not accomplished because diabetic rats require two or three months for cataracts to develop while in galactosemic rats only two weeks are required for cataracts. It was inconvenient to treat rats with the aldose reductase inhibitor for the many months required for the formation of diabetic cataracts. The South American degus turned out to be an excellent animal model for the study of diabetic cataracts. The degus lens has an unusually high level of aldose reductase activity. Thus, when made diabetic the degus developed cataracts within two weeks. With diabetic degus it was possible to show that oral feeding of flavonoids effectively delayed cataract formation. These results provided strong support of the hypothesis that aldose reductase initiated the formation of cataracts in diabetes. This study revealed for the first time that inhibition of aldose reductase not only led to a decrease in the sorbitol accumulation in the lens but also impeded the cataractous process. The cataract formation in diabetes may thus be at least delayed, if not prevented, by the in vivo use of an aldose reductase inhibitor. We have also examined other flavonoids for their ability to inhibit aldose reductase activity in the hope of finding even more potent derivatives than quercitrin. Possibly other flavonoids are effective in still lower doses and are more suitable therapeutically against the diabetic manifestations initiated by polyols.

Significance to Biomedical Research and the Program of the Institute: Cataract is one of the major causes of blindness throughout the world. Even though vision can be corrected by appropriate surgery, loss of vision because of cataracts presents a problem. It is hoped that this type of study on sugar cataracts may serve as a model by which other mechanisms of cataract development can be uncovered, and also provide alternate means of preventing cataracts. The terminal stages of these sugar cataracts may have features common to other forms of cataracts. Even though the initial phase of cataract development may be different in the other forms of cataract, it appears that the terminal stages are quite similar.

Proposed Course: This project will be continued.

NEI Research Program: Cataract - Diabetic Cataract/Congenital, Metabolic, and Genetic Cataract

Experimental Subject or Tissue Source: Rat/Mouse/Degus

Research Objective: Etiology

Publications:

Fukui, H.N., Obazawa, H., and Kinoshita, J.H.: Lens growth in the Nakano mouse. Invest. Ophthalmol. 15: 422-425, 1976.

Varma, S.D., and Kinoshita, J.H.: Topical treatment of galactose cataract Documenta Ophthalmol. Proceed Series. Prog. of Lens Biochemical Research, Dr. W. Junk Publishers p. 305-309, 1976.

Varma, S.D., and Kinoshita, J.H.: Inhibition of lens aldose reductase by flavonoids. Their possible role in the prevention of diabetic cataracts. Biochem. Pharm. 25: 2505-2513.

Kinoshita, J.H., Varma, S.D., and Fukui, H.N.: Aldose reductase in diabetes. Jap. J. Ophthal. 20: 399-410, 1976.

Kinoshita, J.H.: Biochemical basis of cataract formation. Acta Soc. Ophthal. 80: 1362-1371, 1976.

Varma, S.D., Mizuno, A., and Kinoshita, J.H.: Delaying the formation of diabetic cataract with flavonoids. Science 195: 205-206, 1977.

Fukui, H.N., Iwata, S., Epstein, D.L., and Merola, L.O.: Cataractogenic effects of a boron hydride disulfide compound. Invest. Ophthal. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00136-05 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Chemistry and Metabolism of the Lens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Jin H. Kinoshita	Ph.D.	Chief	LVR NEI
Other:	Izumi Kabasawa	M.D.	Visiting Scientist	LVR NEI
	Henry N. Fukui	Ph.D.	Senior Staff Fellow	LVR NEI
	Paul Russell	Ph.D.	Staff Fellow	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Considerable effort has been directed toward developing a tissue culture method for lens epithelial cells. We feel that this approach may aid in the understanding the nature of human congenital cataracts. Thus far, we have been successful in culturing mouse and dog cells. The mouse cell lines have been established. Some success has been achieved with the human lens cell culture.

Further studies have been undertaken to determine the aging effects on the low molecular weight proteins. It is quite apparent that in the aging lens there emerges a new low molecular weight protein.

Project Description:

Objectives: Many aspects of the chemistry and metabolism of normal lens are being studied in order to better understand the significance of the changes that occur in the cataractous process.

Methods Employed: Tissue culture of lens epithelial cells will be undertaken to aid in this study. Procedures for organ culture of the lens will also be used.

Major Findings: We are further exploring the possibility that tissue culture methods may be useful in studying human congenital cataracts. To devise techniques for obtaining cultures of pre-adult and adult lens epithelial cells, lenses from Nakano and normal mice were employed. The Nakano mouse cataract offered an excellent model for this study since the histology of this cataract has been thoroughly investigated. In addition, insight into the biochemical defect has been uncovered. Thus, this mouse cataract provided us the opportunity to test the feasibility of employing tissue culture methods to study a hereditary cataract. Conceivably, if the method is successfully developed it may be useful in the study of human congenital cataracts.

Although the lens epithelial cells from both the normal and Nakano mice grew well in culture with doubling times of about 31 hours, the normal lens cells have twice the clone forming ability compared to Nakano lens cells. After about 16 days in culture large spherical lentoid bodies began to appear. The lentoid bodies were similar to those observed with the chick cell cultures. The lentoid structures were observed in both the normal and Nakano lens cultures. The lentoid structures were observed as early as the tenth day in some lines, and by the thirtieth day, all the cell cultures had lentoid structures. The ability to form lentoid bodies has been retained in cell lines subcultured for over one year.

It has been established that the synthesis of γ crystallin occurred in the fiber cells of the lens rather than the epithelial cells. Fluorescent antibody prepared against mouse γ crystallin reacted with the lentoid structures from both normal and Nakano mouse cells. The immunofluorescence of the lentoid bodies suggested the production of γ crystallin in these structures. The presence of this crystallin indicated the possible differentiation of some of the cells in the lentoid structure.

These results seem to indicate that we have been successful in tissue culture of mouse lens epithelial cells. Many distinctive characteristics of lens epithelial cells are retained in cultures of cells from adult mice. The cells are epithelial in nature even after one year in culture. One of the most unusual feature of these cells is the presence of spherical lentoid bodies. Some cells in the lentoid structure appear similar to the lens fiber cells in that the lack of cellular organelles creates a homogeneous cytoplasm. Antibodies prepared against mouse γ crystallin react with the lentoid bodies, suggesting that some cells in the lentoid structure produced γ crystallin. Since γ crystallin synthesis and loss of cellular organelles are properties of differentiated fiber cells, it appears some cells in tissue

culture retain the ability to express some differentiated characteristics. These cells have consistently shown these characteristics for over one year even with repeated subculture.

Another evidence that culturing of the mouse lens cells does not lead to loss of differentiated traits of the lens is the demonstration that the Nakano cells retain the Na-K ATPase inhibitor. This factor is thought to be responsible for electrolyte imbalance and increased hydration, the changes that precede cataract formation in the lenses of these animals. The inhibitor is found only in the cells from the lenses of the Nakano mice and not from the cells of the normal mice. Since the cells generally are subcultured without difficulty and the doubling time is relatively short, the purification of the inhibitor from these cells may be possible. Since partial differentiation of epithelial cells is possible with the cultures of the mouse lines, other biochemical properties of the cells in culture can also be studied in order to aid the investigation of the cataractous process. The methods are now being used to study material from other species in order to devise an adequate method for use with human congenital cataractous lenses.

Another major effort has been directed to understanding the nature of the low molecular weight proteins. These proteins are the first to disappear in a number of cataracts. The low molecular proteins in the lens are γ crystallins and a low molecular weight beta crystallin called β_s.

In comparing the γ crystallins from old and young bovine lenses that can be recovered by chromatography on Sephadex G-75 column, a cattle lens γ appears which is different from the calf lens γ. The new γ crystallin is found almost exclusively in the cattle lens cortex and not in the nucleus. On the other hand, the γ crystallin present in the cattle lens nucleus is indistinguishable from the calf lens. The γ crystallin along with beta-S isolated from Sephadex G-75 have been purified on cation exchange columns. SDS gel electrophoresis revealed that the molecular weight for cattle lens γ is 24,000, calf lens γ is 20,000 and β_s is 28,000. The N terminal group of cattle lens and calf lens γ is glycine while that of beta-s is masked. Immunodiffusion studies revealed that the cattle lens γ does not cross react with antisera of β_s and calf lens.

A γ crystallin resembling the cattle lens cortex γ is also found in human lens. This γ crystallin increases with age. Its molecular weight is 24,000 and other properties resembles that of the cattle lens γ.

Thus with the aging of the lens there appears to emerge a new γ crystallin in both bovine and human lenses.

Significance to Biomedical Research and the Program of the Institute:
An understanding of the basic chemistry and physiology of the lens is important to provide a more complete understanding of the cataractous process. The age-related change in the gamma crystallins is one of the first demonstrations of the effect of aging of the lens proteins.

Development of a tissue culture procedure of lens epithelial cells may become useful in studying human cataracts. Human congenital cataracts may be studied by this technique provided that epithelial cells from a cataract may be obtained.

Proposed Course: The studies described are being continued.

NEI Research Program: Cataract - The Normal Lens/Congenital, Metabolic, and Genetic Cataract

Experimental Subject or Tissue Source: Bovine/Rat/Mouse

Research Objective: Etiology

Publications:

Fukui, H.N.: The effect of hydrogen peroxide on the rubidium transport of the rat lens. Exp. Eye Res. 23: 595-599, 1976.

Fukui, H.N., Merola, L.O., and Kinoshita, J.H.: The effect of oxidants on the membrane sulfhydryl groups of the lens. Documenta Ophthalmol. 8: 161-169, 1976.

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Kabasawa, I., and Fukui, H.N.: Glycoproteins of the cattle lens plasma membranes. Jap. J. Ophthal. (in press).

Horwitz, J., Kabasawa, I., and Kinoshita, J.H.: Conformation of gamma crystallin of the calf lens. Exp. Eye Res. (in press).

Kabasawa, I., Tsunematsu, Y., Barber, G.W., and Kinoshita, J.H.: Low molecular weight proteins of the bovine lens. Exp. Eye Res. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00179-02 LVR

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Ultrastructural and Biochemical Correlates in the Vertebrate Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Arnold I. Goldman	Ph.D.	Staff Fellow	LVR NEI
Other:	Paul J. O'Brien	Ph.D.	Staff Scientist	LVR NEI
	Eileen Masterson	Ph.D.	Postdoctoral Fellow	LVR NEI
	Gerald Chader	Ph.D.	Staff Scientist	LVR NEI
	Paul Tierstein	B.S.	Summer Student	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Biochemistry

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.6	1.4	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Studies are being conducted on the process of phagocytosis of outer segment membranes by the pigment epithelium, with emphasis on control mechanisms of this process. Two separate experimental methodologies have been developed to study this problem. In the first, bovine retinas have been radioactively labeled and the outer segments separated and fed to chick pigment epithelium cell cultures. The time course of ingestion of the outer segments can be monitored with a liquid scintillation counter and has been verified by electron microscopy and histochemistry. By placing appropriate sugars or hormones with the outer segments, the effect of these agents on phagocytosis can be quantified. In the second approach, normal and dystrophic rat eyecups are incubated in vitro in a solution containing radioactive sugars and/or melatonin. Both autoradiography and phagosome counts are made to see if the sugars are instrumental in preparing the outer segments for shedding, and to see if the melatonin plays a role in controlling this process.

Project Description:

Objectives: Biological functions of the photoreceptor have been previously studied by autoradiographic, biochemical and histochemical methods. Each of these methods has limitations for the achievement of certainty. However, when these methods are used jointly, the correlations become highly significant. This project was designed to bring multiple disciplines to bear on major problems concerning photoreceptor function, especially as regards the renewal of the outer segment and the process of shedding and phagocytosis of outer segment discs.

Methods Employed: Biochemical methods such as isolation and incubation of rod outer segments, column chromatography and scintillation counting were used to obtain an overall impression of the biochemical events associated with disc shedding and phagocytosis. This work was correlated with autoradiography of ^{3}H-galactose or fucose in the rat to see if there was localization of the transfer of these sugars. Tissue culture techniques allowed the use of chick PE cultures to serve as a practical quantitative assay of phagocytosis. Electron microscopic histochemistry was used to verify the biochemical findings with the tissue culture system.

Major Findings: Rhodopsin in the outer segments of the photoreceptors will take up both fucose and galactose. Preliminary evidence suggests that the uptake of the sugars is greatest at the tips of the outer segments, implying that they are somehow involved in preparing the outer segments for phagocytosis. Eyecup preparations incubated with melatonin show little shedding, while those incubated without melatonin shed a large portion of their outer segment tips. If melatonin is given for the first part of the incubation and the incubation is continued in its absence, shedding is still further increased. These preliminary results imply that melatonin inhibits disc shedding while it "primes" the outer segments for later shedding. The tissue culture assay of phagocytosis has proven to be a reliable and quantitative method. Both galactose and mannose in solution appear to stimulate phagocytosis, as does fucose to a lesser degree. Cyclic AMP inhibits phagocytosis measureably.

Significance to Biomedical Research and the Program of the Institute: The mechanisms of regulation of shedding of discs and their subsequent phagocytosis by the pigment epithelium is crucial in the understanding of photoreceptor renewal processes. The knowledge gained in this way can be instrumental in developing treatments for various retinal diseases. Autoradiography is a delicate probe into many of these mechanisms, and the use of the tissue culture technique is a powerful new tool which should yield important new information about phagocytosis.

Proposed Course: Autoradiography will be continued with and without melatonin on both the light and electron microscopic level. Both normal and dystrophic rats will be used to see if there is a difference in the response of these animals, and to use the debris of the dystrophic animals to physically separate the outer segment tips from the pigment epithelium. Since the

pigment epithelium labels more heavily than the outer segments, this separation should make it easier to recognize an accumulation of label at the outer segment tips. The tissue culture system will be used extensively to test various agents on the phagocytic response. Lectins will be used to see if the blockage of specific sugar moieties will inhibit phagocytosis.

NEI Research Program: Retinal and Choroidal Diseases - Developmental and Hereditary Disorders/Visual Cells and Pigment Epithelium

Experimental Subject or Tissue Source: Bovine/Rat/Chick

Research Objective: Etiology

Publications:

Goldman, A.I., Ham, W.T., Jr., and Mueller, H.A.: Ocular damage thresholds and mechanisms for ultrashort pulses of both visible and infrared laser radiation in the rhesus monkey. Exp. Eye Res. 24: 45-56, 1977.

Geeraets, W.J., Geeraets, R., and Goldman, A.I.: Elektromagnetische bestrahlungsvertletzungen der netzhaut (Electromagnetic radiation damage to the retina), Albrecht v. Graefes Archiv f. Klin. Exp. Ophthalmologie 200: 263-278, 1976.

Geeraets, W.J., Ham, W.T., Jr., Geeraets, R., and Goldman, A.I.: Photochemical, thermal, and non-linear effects of retinal irradiation, In L'Esperance Francis, (ed.), Current Diagnosis and Management of Chorioretinal Diseases. St. Louis, Mosby, 1977, pp. 9-24.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00135-05 LVR

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Biochemical Structure of Retina and Pigment Epithelium in Health and Disease

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Helen H. Hess	M.D.	Research Medical Officer	LVR NEI
Other:	Julia E. Derr	B.A.	Biologist	LVR NEI

COOPERATING UNITS (if any)

Clinical Branch, NEI; Division of Research Services, Veterinary Resources Branch; Medical Neurology Branch, NINCDS; American HistoLabs, Inc. (contract)

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Biochemistry

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.05	1.25	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The broad aim of the project is to study the biochemical composition of retina, pigment epithelium and rod outer segments in normal circumstances and in retinal and choroidal diseases of experimental or genetic origin. Topics of current interest are: (a) concentration and distribution of inorganic constituents; (b) possible involvement of calcium, zinc and copper in retinal and choroidal diseases;)c) ultrastructural localization and physiological function of calcium in retina, pigment epithelium, and choroid; (d) study of hybrids of RCS and spontaneously hypertensive rats to determine whether the slow onset type of retinal degeneration seen in the latter is inherited at the rdy or another gene locus or is due to light damage in an albino animal.

PHS-6040

Project Description:

Objectives: To study the biochemical composition of retinal photoreceptor, neuronal, glial, and pigment epithelial cells in health and disease, and to explore possibilities for prevention or therapy of retinal and/or choroidal disease when a biochemical abnormality has been identified; diseases in which pigment epithelium (PE) is involved are of particular interest.

Methods Employed: Retinas, isolated rod outer segments (ROS), and PE of frogs and rats are being analyzed. Samples of plasma or serum as well as urine are being studied in rodent models and human cases of retinal degeneration. Methods include flameless atomic absorption spectroscopy, microscopy, and a number of standard biochemical laboratory techniques.

Major Findings: I. Studies of trace elements: Last year the distribution of nine inorganic elements was studied in PE, ROS, and retina of frogs. This year two trace metals were chosen for further study in rodents and in humans with retinal disease (Zn and Cu).

(a) Zinc in blood plasma of RCS dystrophic and congenic control RCS rats: Rats have uniquely high levels of Zn in milk and plasma during development. We have found that during the first postnatal week, RCS rat plasma Zn is two to four times as high as in the adult RCS rat. A time lag in breeding of RCS control rats has delayed making direct comparisons at the required ages (1,7,14,18,21, and 22 days) in mutant and control animals. The postnatal developmental pattern of plasma Zn in RCS mutants, however, is not identical with that in Osborne-Mendel albino rats. Plasma Zn levels of female rats are lower than in males after two weeks of age, when estrogen begins to be produced. The lability of plasma Zn in the developmental period is illustrated by our observation that a 50% drop in plasma Zn on day 22 can occur after milk has been stopped abruptly at weaning on day 21 and lab chow begun.

(b) Histopathological study of eyes of copper deficient mutant mice, an animal model of Menkes disease: five alleles at the mottled locus on the X chromosome of the mouse are responsible for mutations of Cu deficiency. Dapple and Tortoise mutants die before birth. Brindle mutants survive to 12-14 days, and up to 25 days with Cu injections. Blotchy and Crinkled mutants live 25 days, or longer if high copper diets are given.

NIH colony stocks of Brindle were previously supplied on a C3H background and contained the rd gene for retinal degeneration (satisfactory for investigators not studying retina). We manipulated these animals genetically to remove the rd gene and place the Brindle gene in a C57 Black 6 background for study of possible ocular pathology (in collaboration with Dr. Kitty Smith, Division of Research Services, Veterinary Resources Branch). Stained sections of 12-14 day old eyes showed retardation of retina development, with incomplete development of photoreceptor outer segments. In consultation with Dr. T. Kuwabara, pigment epithelial cells of retina and iris were examined and appeared to contain vacuoles or microcysts, especially those of the iris. This young mutant did not show optic nerve atrophy and loss of ganglion cells.

(c) Urinary excretion of trace metals in humans with hereditary retinal

degeneration: Recently (Gahlot, 1976) the amount of Cu excreted in a 24 hour urine specimen of patients with primary retinitis pigmentosa (RP) was reported to be six-fold normal. Normals were in a range expected from published work. To investigate this report, we have initiated a study of 24 hour Cu; for comparison, Zn was included. A masked study was designed, with Dr. D. Bergsma (Clinical Branch, NEI) selecting the types of patients included, and the analyses were done by a second person not informed of the patient type prior to assay. Results for Cu in two normals and in four cases of retinal degeneration (two with primary recessive RP, one with autosomal dominant RP, and one with Usher's syndrome) were all in the normal range reported in the literature. However, values for Zn in the three cases of RP were greater than the extreme of the normal range reported in the literature, while values in the case of Usher's syndrome and in the two normals were within the normal range.

II. Ultrastructural localization of Ca in retina and PE: The EM study to localize Ca by use of the potassium pyroantimonate technique (in collaboration with Dr. M.L. Fishman, Clinical Branch, NEI; and M.A. Oberc and Dr. W.K. Engel, NINCDS) was completed. Optimum preservation was obtained with 2% K pyroantimonate in 1% osmium tetroxide at pH 9.2. With this fixative, electron-dense precipitate was found within the discs of the ROS; as a dense band of granules along Bruch's membrane; in the extracellular space of the choroid; and to some extent within the nuclei and mitochondria of all cells. The precipitate was identified as Ca antimonate by use of EGTA prechelation and with the EMMA-4 electron microprobe.

This is the first time calcium has been demonstrated within ROS discs and in normal Bruch's membrane.

III. Studies of hybrid rats from two strains with retinal degeneration: Last year nine black hooded progeny from crossing tan hooded RCS dystrophic rats with albino spontaneously hypertensive (SH) rats were reported to have normal photoreceptors at one year of age, as shown by microscopy of fresh and fixed, stained specimens. Similarly, we have studied 19 more of the same F_1 progeny, now two years of age, with the same result. Since retinal degeneration occurs at a few weeks of age in the RCS rat and at 6-12 months of age in the SH rat, the hybrid (even though pigmented) would be expected to show changes before 12 months (and certainly by two years) if the rdy gene were present in the SH genome, or if a different abnormal gene were present at the same locus.

From this study it seems apparent that the SH rat has no abnormal gene at the rdy locus. This suggests that albino rats such as SH, Osborne-Mendel and Sprague-Dawley rats in which slow onset retinal degeneration has been described may have these changes on the basis of light damage. To help rule out this etiological factor and to explore whether the SH rat may have an abnormal gene at a different locus, F_2 generation animals ($F_1 \times F_1$) were produced and will be one year of age in September 1977. The color ratios are approximately two black hood to one tan hood to one albino. Among black hooded animals, light damage does not occur and any excessive appearance of retinal dystrophy beyond the ratio of one dystrophic to three normals would suggest that a second gene for retinal degeneration is involved (collaboration with Dr. C. Hansen, geneticist, Division of Research Services, Veterinary Resources Branch).

Significance to Biomedical Research and the Program of the Institute: Zinc affects many structural proteins and enzymes of significance in PE and retina and is a plasma membrane active agent whose concentration can influence phagocytosis. Plasma Zn varies rapidly with nutritional and hormonal changes, especially in the developing animal. Since plasma albumin, a major Zn carrier, is low and the blood-ocular barrier not yet established, this lability may be significant in pathology of the developing eye. Genetic and nutritional factors are known to interact in Zn and Cu metabolism. When an error in regulation of the metabolism of such trace metals occurs, nutritional methods of treatment may be of some avail.

In one case of Menkes' disease, Wray, Kuwabara and Sanderson (1976) confirmed the loss of retinal ganglion cells and optic atrophy first reported in one case by Seelenfreund, et al. (1968), but they did not find vacuolation of the iris PE which had been seen by Seelenfreund et al. Iris PE vacuole formation may occur subsequent to terminal therapeutics (IV fluids, drugs), however, and its relevance to copper deficiency has not been established. If microcysts of PE can be shown consistently in an animal model like the Brindle mouse, this finding would attain more significance. Varied findings in pathology of Menkes' disease could result not only from study of different stages of disease but also perhaps from existence of several alleles governing Cu, as in the case of mice.

Our studies support the specificity of the K pyroantimonate technique in showing the ultrastructural localization of Ca in retina and related tissues. Our demonstration of Ca within ROS discs is consistent with some role for Ca in either mediating the photoexcitation process or in dark and light adaptation, or both. We showed previously that dark-adapted frog ROS contain Ca in a ratio of about 0.25 moles/mole rhodopsin. We also reported that light adaptation in vivo increased the Ca content of ROS. The Ca that may act as a mediator of photoexcitation is thus probably a very small fraction of the total Ca present in ROS in either the dark- or light-adapted state.

The large amount of extracellular Ca in Bruch's membrane indicates numerous Ca-binding sites. This may be related to the frequency with which abnormal calcification occurs in this area, as in angioid streaks, drusen, and macular degeneration. These findings suggest a need for further study of the distribution of Ca in physiologic and pathologic processes.

If the SH rat has a separate gene for slow onset retinal degeneration, this gene could be placed on a pigmented background to give an animal model more nearly analogous to human retinitis pigmentosa, to supplement the fast onset model of the RCS strain.

Proposed Course: Developmental analyses of Zn (and Cu) in plasma of RCS mutant and RCS control rats will be completed. Further studies of Zn, Cu, Ca and enzymes or proteins to which they are related will be pursued in PE cells prepared by frozen sectioning and tissue culture, as well as in retina. Additional specimens of Cu deficient mouse mutants will be examined for comparison with pathology of Menkes' disease. A larger group of specimens

of urine from different categories of retinal degeneration will be analyzed to determine whether hyperzincuria is of any significance in such diseases and to check further on levels of copper. The F_2 hybrids from the cross of RCS and SH rats will be examined at 12 months and later to determine retina integrity by ERG (with collaboration of Dr. P. Gouras, Physiology Section, LVR) and in stained sections.

NEI Research Program: Retinal and Choroidal Diseases - Developmental and Hereditary Disorders/**Visual Cells and Pigment Epithelium.**

Experimental Subject or Tissue Source: Frog/Rat/Human

Research Objective: Etiology

Publications:

Whikehart, D.R. and Hess, H.H.: Properties of liposomes with a phospholipid ratio similar to that of retinal rod outer segment membranes. Interaction with opsin and other proteins. Exp. Eye Res. 24: 279-289, 1977.

Fishman, M.L., Oberc, M.A., Hess, H.H., and Engel, W.K.: Ultrastructural demonstration of calcium in retina, retinal pigment epithelium, and choroid. Exp. Eye Res. 24: 341-353, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00008-06 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Chemistry of Rhodopsin

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marc S. Lewis	Ph.D.	Research Chemist	LVR NEI
Other:	Hitoshi Shichi	Ph.D.	Research Chemist	LVR NEI
	Gerald Chader	Ph.D.	Head, Section on Retinal and Corneal Metabolism	LVR NEI
	Barbara Wiggert	Ph.D.	Staff Fellow	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section of Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It has been the long range objective of this project to study the rhodopsin molecule and other molecules, such as retinol and retinoic acid which are involved in the visual process in order to attempt to elucidate further information regarding the relationships between their structures and their functions. The areas of current interest are studies on the various forms of rhodopsin which can be complexed with digitonin and studies on the interactions of retinol and retinoic acid with receptor sites in the retina and the cornea.

Project Description:

Objectives: To study the structural and functional aspects of the rhodopsin molecule and to study the interaction of retinol and retinoic acid with receptor sites in the retina and cornea.

Methods Employed: Rhodopsin has been isolated as a rhodopsin-digitonin complex from bovine retinas, the various forms of the complex have been isolated chromatographically and studied by means of sedimentation-equilibrium in the analytical ultracentrifuge, and the data analyzed by mathematical modelling techniques using the MLAB system on the DEC-10 computer. Retinol and retinoic acid receptors from retina and cornea have been isolated and the ligand interactions studied by sucrose density gradient ultracentrifugation, gel filtration, spectroscopy, and electrophoresis. The binding data was analysed by mathematical modelling techniques using the MLAB system on the DEC-10 computer.

Major Findings: Two forms of rhodopsin complexed with digitonin have been isolated. Type I had a molecular weight of 259000 when unbleached and 226000 following bleaching. Type II had a molecular weight of 191000 when unbleached and 186000 following bleaching. The two types show quite different bleaching and regeneration characteristics, Type I bleaching slowly and regenerating rapidly, and Type II having the reverse properties. The present evidence tends to indicate that the Type I complex is a dimer of rhodopsin complexed with digitonin and that the Type II complex is a monomer of rhodopsin complexed with digitonin. The evidence here, while preliminary, is causing us to consider the possibility that a monomer-dimer transition may be involved in the bleaching-regeneration processes of rhodopsin.

Specific soluble receptors for retinol and retinoic acid have been found in retina, pigment epithelium and in cornea. Two different receptors for retinol have been found in the retinas of most species. One of these was not seen in the retina of a patient with retinitis pigmentosa and during early fetal development in the cow, thus indicating that this particular receptor type is associated with photoreceptor outer segments in vivo. The molecular weights of the receptors and the association constants for the binding of retinol and retinoic acid to the receptors were determined. These receptors may be involved in the normal development and the transport of vitamin A in ocular tissues.

Significance to Biomedical Research and the Program of the Institute: The studies on rhodopsin are relevant to an understanding of the basic biochemical mechanisms which are involved in normal and pathological aspects of scotopic vision. The studies on vitamin A receptors are relevant to the normal and pathological aspects of development and function of the retina, pigment epithelium and cornea, and as such are of significance in such diseases as retinal dystrophy and keratomalacia which appear to involve vitamin A.

Proposed Course: This project is being terminated since the principal investigator is transferring from the National Eye Institute to the Division of Research Services. Plans have been made for continuation of this research on a collaborative basis.

NEI Research Program: Retinal and Choroidal Diseases - Visual Cells and Pigment Epithelium

Experimental Subject or Tissue Source: Cow/Rat/Monkey/Pig/Chicken/Human

Research Objective: Etiology

Publications:

Wiggert, B., Bergsma, D., Helmsen, R., Lewis, M., and Chader, G.: Retinol receptors in corneal epithelium, stroma and endothelium. Biochim. Biophys. Acta 491: 104-113, 1977.

Wiggert, B., Bergsma, D., Lewis, M., Abe, T., and Chader, G.: Vitamin A receptors: Characteristics of retinol binding in chick retina and pigment epithelium. Biochim. Biophys. Acta (in press).

Wiggert, B., Bergsma, D., Lewis, M., and Chader, G.: Vitamin A receptors: Retinol binding in neural retina and pigment epithelium. J. Neurochemistry (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER ZO1 EY 00009-06 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Physical Chemistry of Model Gel Systems

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Marc S. Lewis	Ph.D.	Research Chemist	LVR NEI
Other:	Jules A. Gladner	Ph.D.	Research Chemist	LBC NIAMDD
	S.I. Chung	Ph.D.	Research Chemist	LB NIDR
	Yutaka Shizuta	M.D.,Ph.D.	Visiting Scientist	LMB NCI
	Peter Davies	M.D.,Ph.D.	Research Associate	LMB NCI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long-range objective of this project to study various biological systems which are either actually involved in the visual process or which may serve as models for such systems which are relevant to the transparency or opacity of ophthalmic tissues. Of current interest are the structure and cross-linking mechanisms of fibrinogen and the physical, chemical, and biological properties of the smooth muscle protein filamin.

Project Description:

Objectives: To study the physical and chemical parameters of model systems which are pertinent for transparency or opacity of gel systems or which in any way may be of significance in the biochemistry of vision.

Methods Employed: The usual methods of protein preparation, fractionation, purification and characterization have been employed. In this laboratory particular emphasis has been given to analytical ultracentrifugation and to computer techniques for model simulation, data reduction, and systems analysis as being the most effective means of studying systems of interacting and non-interacting macromolecules.

Major Findings: Further work has been done on the most primitive of the vertebrate fibrinogens, lamprey fibrinogen. Ultracentrifugal analysis of the native molecule in aqueous buffer gave a value for the molecular weight of 352000 and SDS gel electrophoresis analysis gave values for the molecular weights of the constituent chains such that a structure of one alpha chain, two beta chains, and two gamma chains was postulated. Following isolation of the constituent chains their molecular weights in 6 M guanidine hydrochloride were measured in the ultracentrifuge, and molecular weights markedly lower than those obtained in the gel studies were obtained when a value for the partial specific volume of 0.704 was used. This value was computed from the amino acid composition, taking into account possible changes in hydration and guanidine binding. In order to help resolve this issue, the molecular weight of intact lamprey fibrinogen was measured in 6M guanidine buffer, and a value of 281000 was obtained with the partial specific volume value of 0.704. In order to obtain the same value for the molecular weight as that obtained in aqueous buffer, it was necessary to use a value of 0.736 for the partial specific volume, thus indicating that the assumptions regarding the extent of hydration or guanidine binding were not valid. This property does not appear to be unique to lamprey fibrinogen, since bovine fibrinogen gave a value of 274000 for the molecular weight in 6M guanidine buffer when the computed value of 0.720 was used for the partial specific volume. Since the assumptions with regard to hydration and guanidine binding are for typical globular proteins, it is not particularly surprising that they are not necessarily valid for a markedly assymetrical protein like fibrinogen. Individual chain molecular weight values obtained were 109000, 73000, and 48500 for the alpha, beta, and gamma chains respectively, adding up to a total of 352000 for the molecular weight of the whole molecule having the postulated structure. Further verification was obtained by chromatography on Sepharose using 6M guanidine buffer where values of 115000, 76000, and 50000 were obtained for the molecular weights of the respective chains. We now feel that the unique structure of lamprey fibrinogen has been suitably established and are proceeding with the preparation of a manuscript describing this work.

Studies were initiated on the properties of the protein filamin which has been found in smooth muscle as well as in non-muscle cells such as macrophages and fibroblastic cells. The filamin used in this work was isolated from chicken gizzard. Sedimentation-equilibrium studies gave a value of 498000 for the molecular weight of the native protein in 50 mM phosphate, 0.1 M NaCl,

pH 7.5 buffer. The value of 0.734 for the partial specific volume was computed from the amino acid composition. A value of 8.86 S was obtained for the sedimentation coefficient in the same buffer, and a frictional ratio of 2.32 was calculated, indicating a significant degree of asymmetry. Filamin migrates as a single sharp band on SDS gel electrophoresis, and has a apparent molecular weight of 240000, thus indicating that it is normally composed of two identical subunits. Filamin is a soluble protein and under a variety of conditions tested does not by itself form filaments or precipitate from solution. However, filamin binds very strongly to rabbit muscle F-actin, and the complex is readily sedimented to yield a gelatinous pellet containing these proteins. Filamin has been demonstrated to undergo a variety of very complex associations with itself depending upon temperature, salt, ionic strength, and pH. For example, in 0.6 M KCl at pH 7.4, it appears to be a monomer-dimer-tetramer association at low temperatures, shifting first to a monomer-tetramer association and then to an ill-defined monomer-n-mer association as the temperature is increased. The reversibility of the association also appears to be a function of the environmental conditions. As a result, it has been difficult to clearly define the aggregation status of filamin under physiological conditions except to state that it obviously exists as a complex with actin. The molecular weight of this complex is of such a magnitude as to preclude study by conventional methods, but studies on filamin fragments produced by enzymatic cleavage appear promising in terms of delineating which portions of the filamin molecule are involved in actin binding, which are involved in self-association, and may also yield significant information concerning the nature of these interactions.

Significance to Biomedical Research and the Program of the Institute: The studies on fibrinogen represent a contribution to the general area of inter- and intra-molecular cross-linking and the formation of gels. More specifically, because of the role of fibrinogen in blood clotting and wound healing, it is relevant to the surgical treatment of ophthalmic disorders. The role of filamin in the eye has as yet to be elucidated, but it would be reasonable to expect that it is present to a significant extent in some ophthalmic tissues, and that future studies will determine what function it might have.

Proposed Course: This project is being terminated since the principal investigator is transferring from the National Eye Institute to the Division of Research Services. The studies on lamprey fibrinogen have been completed. The studies on filamin will be resumed as a new project.

NEI Research Program: Corneal Diseases - Corneal Transplantation and Stromal Injury and Repair

Experimental Subject or Tissue Source: Lamprey/Chicken

Research Objective: Etiology

Publications:

Shizuta, Y., Shizuta, H., Gallo, M., Davies, P., Pasten, I.,and Lewis M.S.: Purification and properties of filamin, an actin binding protein from chicken gizzard. J. Biol. Chem. 251: 6562-6567, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00004-03 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Membrane Biology of the Visual Process

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hitoshi Shichi	Ph.D.	Research Chemist	LVR NEI
	Alois J. Adams	Ph.D.	Post Doctoral	LVR NEI
Other:	Robert L. Somers	B.S.	Chemist	LVR NEI
	Consuelo G. Muellenberg	B.A.	Biologist	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	1.5	2.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) A method is being developed for extraction and purification of protein kinases associated with rod membranes, one that phosphorylates rhodopsin with ATP and the other that phosphorylates phosvitin. 2) Three isochromic forms of rhodopsin have been separated and purified. These forms contain different amounts of phospholipid and demonstrate different bleaching kinetics.

Project Description:

Objectives: The overall objectives of this project are to investigate the light-dark adaptation processes of the retina by means of modern techniques of biochemistry and membrane biology. More specifically, these are (1) identification of a sequence of molecular events initiated by absorption of photons and leading to visual transduction (light process) and (2) elucidation of the biochemical mechanism of regeneration of the photosensitivity of photoreceptor membranes (dark process). The investigations presented in this report deal with two aspects of the visual pigment rhodopsin, i.e. (a) modulation of rhodopsin bleaching kinetics by associated phospholipid, and (b) the phosphorylation of opsin.

Methods Employed: Biochemical methods such as centrifugation, column chromatography, spectroscopic analysis and radioisotope assay. Low temperature spectroscopy and fast kinetics measurements after a flash photolysis of rhodopsin.

Major Findings:

(1) Isochromic forms of rhodopsin

On the basis of kinetic studies of rhodopsin bleaching in digitonin, the existence of multiforms of rhodopsin and of subsequent bleaching intermediates in this detergent has been previously suggested. Purification of digitonin-extracted rhodopsin on ECTEOLA-cellulose results in the separation of three isochromic forms of rhodopsin (Fr. I, II and III). Fr. I and Fr. II constitute about 45% and 40% of the total rhodopsin purified, respectively. The three fractions of rhodopsin are indistinguishable in their spectral properties with the characteristic absorption bands (α at 498 nm, β at 350 nm, and γ at 278 nm). They differ in phospholipid content; the molar ratio of phospholipid to rhodopsin is 14 for Fr. I and 64 for Fr. II. Delipidation converts Fr. II to Fr I rhodopsin and Fr. I is transformed back into Fr. II rhodopsin by reassociation with phospholipid. From these results we conclude that the multiple forms of rhodopsin in digitonin result from nonuniform association of phospholipid with opsin.

In order to investigate the thermal stability of the intermediates formed after flash bleaching of rhodopsin, low-temperature spectroscopic measurements have been made on Fr. 1 and Fr 2 rhodopsins. On the basis of thermal stability, both bathorhodopsin and lumirhodopsin are identical whether they are derived from Fr I or Fr. 2. However, a comparison of these intermediates between digitonin extracts and rod membranes shows that the stability of bathorhodopsin remains unaffected whether it be formed in rods or in digitonin and that lumirhodopsin, on the other hand, becomes more labile when it is formed in digitonin than in rods. Since a close correlation exists between spectrally determined thermal stability and opsin conformational stability, the above results are taken to suggest that the first appreciable conformational change of opsin will take place at lumirhodopsin level but not at bathorhodopsin level. The rate of metarhodopsin I decay is markedly affected by the phospholipid content of rhodopsin. The metarhodopsin I decay rate determined at

11°C is $0_{-1}11$ sec^{-1} for Fr. 1 (14 moles phospholipid per mole rhodopsin) and 5.20 sec^{-1} for Fr. 2 (64 moles phospholipid per mole rhodopsin). Because of the low phospholipid content, the decay of metarhodopsin I derived from Fr. 1 will be affected by digitonin to a greater extent than that derived from Fr. 2. In other words, the digitonin micelle associated with rhodopsin will affect the decay rate by producing such a rigid environment that opsin conformational change associated with metarhodopsin I decay is prevented. The decay of metarhodopsin III is also affected by the amount of phospholipid associated with opsin. The slower decay rate of metarhodopsin III derived from Fr. 1 can be explained also by assuming the stabilization of opsin conformation by digitonin. Phospholipid unsaturation is recently shown to be essential for the metarhodopsin I to metarhodopsin II conversion. A suggestion is then made that a minimum phospholipid biplayer fluidity is necessary to allow opsin to undergo conformational changes associated with the transition. Thus, the present investigation supports this suggestion by demonstrating that thermal intermediates placed in the rigid (i.e. low fluidity) environment of digitonin micelle decay more slowly than those in fluid environment.

(2) Phosphorylation of opsin

Rod outer segments seem to contain several protein kinases. At least two phosvitin kinases (40,000 d. and 170,000 d.) and one rhodopsin kinase (80,000 d.) can be separated by a column-chromatographic method. These kinases show different solubilities in aqueous salt solutions. Activities of these enzymes are not stimulated by cyclic nucleotides. During the course of purification of the kinases, a nucleotide-containing protein (20,000 d.) has been separated.

Significance to Biomedical Research and the Program of the Institute: The effect of phospholipid (as well as digitonin) on the decay rate of metarhodopsin I and III can point to the importance of membrane fluidity of the environment surrounding opsin. Since the fluidity of rod membranes is provided by unsaturated acyl chains of phospholipid, the importance of essential fatty acids in foodstuffs for maintaining normal vision is emphasized.

Proposed Course: This project will be continued.

NEI Research Program: Retinal and Choroidal Diseases - Visual Cells and Pigment Epithelium

Experimental Subject or Tissue Source: Bovine/Frog

Research Objective: Etiology

Publications:

Shichi, H.: Molecular biology of the visual process. In Siegel, G.J., Albers, R.W., Katzman, R. and Agranoff, B.W. (eds.): Basic Neurochemistry. Boston, Little, Brown & Co., pp. 148-163, 1976.

Shichi, H., Muellenberg, C.G., Harosi, F.I., and Somers, R.L.: Isolation of three isochromic forms of rhodopsin in digitonin. Vision Res. 17: 633-636, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00007-03 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Molecular Pharmacology of the Eye

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Hitoshi Shichi	Ph.D.	Research Chemist	LVR NEI
Other:	Daniel W. Nebert	M.D.	Chief	DPB NICHD
	Douglas E. Gaasterland	M.D.	Senior Staff Ophthalmologist	CB NEI

COOPERATING UNITS (if any)

National Institute of Child Health and Human Development

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.5	0.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Following an intraperitoneal injection of acetaminophen into polycyclic hydrocarbon-responsive mice in which hepatic aryl hydrocarbon hydroxylase (AHH) activity has been induced by pretreatment with polycyclic hydrocarbons, lenticular opacification develops in a few hours. The opacity consists of a thin layer anterior to the equatorial cortex. Acetaminophen metabolites bind to the lens but do not lower the glutathione level. These observations suggest that lenticular opacification may be caused by binding of acetaminophen metabolites to the lens cells in the anterior portion and thus disrupting permeability properties of the cells. Since lenticular opacification develops when mice are pretreated with 3-methylcholanthrene (cytochrome P_1 450 inducer) but not with phenobarbital (cytochrome P 450 inducer), acetaminophen metabolites that affect the lens must be produced by the cytochrome P_1 450-dependent AHH system.

Project Description:

Objectives: We have previously demonstrated that aryl hydrocarbon hydroxylase (AHH) induction in the eye (pigmented epithelium) and liver of polycyclic hydrocarbon-responsive mouse strains is apparently under the same genetic regulation.

In this work we report that administration of large doses of acetaminophen to mice in which AHH has been induced by pretreatment with polycyclic hydrocarbons (e.g. 3-methylcholanthrene) causes development of lenticular opacification in a few hours. A possible mechanism of the lens opacity formation is investigated.

Methods Employed: Mice were injected intraperitoneally with 3-methylcholanthrene to induce AHH activity for 48 hours. Acetaminophen was then injected intraperitoneally into pretreated mice and development of lenticular opacification was examined in vivo as well as in vitro. Eyes were fixed and subjected to histochemical examination. Glutathione levels of lens and liver were determined by a spectroscopic method. Covalent binding of acetaminophen metabolites to lens, liver and other tissues was studied with ^{3}H-acetaminophen.

Major Findings: 1) Acetaminophen-induced ocular opacity in mice under the conditions is unique in that it develops very rapidly (ca 6 hours). 2) Ocular opacity is localized in the anterior portion of the lens. This indicates that toxic acetaminophen metabolites reach the lens in aqueous humor circulation. 3) Cataract develops only in 3-methylcholanthrene-pretreated mice; untreated mice do not show opacity development. 4) Opacity develops only in AHH-inducible strains (e.g. C57/BL 6) and not in non-inducible strains of mice (e.g. DBA/2). 5) Cataract develops when responsive mice were pretreated with 3-methylcholanthrene (cytochrome P_1 450 inducer); pretreatment with phenobarbital (cytochrome P 450 inducer) does not cause opacity. Therefore, acetaminophen metabolites that cause lens opacity are formed by cytochrome P_1 450-dependent AHH system. 6) The glutathione level of lens remains virtually unchanged during opacity development; liver glutathione level is markedly reduced. 7) Acetaminophen metabolites are bound covalently to the lens. On a basis of metabolites bound per mg protein, the levels of binding to lens and liver are almost comparable. In the lens, metabolites therefore seem to bind preferentially to cellular membranes over glutathione. 8) From these results it is suggested that (i) cataract development in mice treated with polycyclic hydrocarbons and acetaminophen is closely related to AHH inducibility, (ii) cataractogenic agents are probably formed by hydroxylation of acetaminophen by hepatic cytochrome P_1 450-dependent AHH system, (iii) acetaminophen metabolites that bind covalently to lens cells probably modify permeability properties of the cells to cause cataract, and (iv) while the lens is affected by acetaminophen metabolites, both retina and pigment epithelium demonstrate little cellular degeneration. This may be attributed to the detoxifying activity of the drug metabolizing system of pigmented epithelium which we have previously elucidated.

Significance to Biomedical Research and the Program of the Institute: The present study on lenticular opacification caused by acetaminophen in mice shows that AHH-inducible strains are particularly susceptible to the toxic effect of acetaminophen on the lens. Acetaminophen is a widely used analgesic-antipyretic agent. It is contained in common drugs such as Excedrin and Tylenol. As much as 800 mg of acetaminophen per kg body weight is administered to humans for treatment of certain cases of arthritis. The present result on experimental animals raises a possibility that high doses of acetaminophen administered to the polycyclic hydrocarbon responsive (i.e. AHH inducible) type of patients, especially on a prolonged basis, might cause development of lenticular opacification.

Proposed Course: This project will be continued.

NEI Research Program: Retinal and Choroidal Diseases - Visual Cells and Pigment Epithelium/Special Areas of Future Interest (Toxic and Environmental Disorders) Cataract - Cataract Induced by Drugs, Radiation, and Secondary to Other Eye Disorders

Experimental Subject or Tissue Source: Mouse

Research Objective: Etiology, Diagnosis

Publications:

Shichi, H., Tsunematsu, Y., and Nebert, D.W.: Aryl hydrocarbon hydroxylase induction in retinal pigmented epithelium: Possible association of genetic differences in a drug metabolizing enzyme with retinal degeneration. Exp. Eye Res. 23: 165-176, 1976.

Shichi, H., and Nebert, D.W.: Drug metabolism in ocular tissues. In Gram, T.E. (ed.): Extrahepatic Drug Metabolism Spectrum Publications, Inc. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00138-05 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

The Visual Cell: Process of Photoexcitation and Restoration

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	S. Yoshikami	Research Biologist	LVR NEI
Other:	G.N. Noll	Visiting Associate	LVR NEI
	W.A. Hagins	Chief, Section Membrane Biophysics	NIAMDD

COOPERATING UNITS (if any)

National Institute of Arthritis, Metabolism and Digestive Diseases

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Biochemistry

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.5	2.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

There is considerable evidence favoring our notion that calcium plays a central role in the initiation of vision. A definitive test for this hypothesis is a measurement of transient light stimulated calcium activity changes in the visual receptor cell. The properties of a calcium sensitive dye, dichlorophosphonazo III, have been studied and found suitable for this purpose. A rapid multi-wavelength spectrometer was built to measure ionic activity changes reported by dichlorophosphonazo III in thin tissues like the retina.

Isolated retinas can function as light detectors but cannot regenerate visual pigments. We have invented a method using liposomes to permit pigment regeneration in such retinas. This technique is applicable to general practice of introducing large amounts of water insoluble chemicals into live tissues. Using this method, we are studying the biochemical pathway of retinol in the visual process and discovered among other things the retinol oxidation enzyme in the retina is specific for a given stereoisomer.

Project Description:

Objectives: To study the nature of the visual cell and determine its physical and chemical means of initiating and sustaining the phenomenon of vision.

Methods Employed: This study employs measurements of the electrical, biochemical, anatomical and metabolic properties of the retina and associated ocular tissues.

Major Findings: I. We (Hagins and Yoshikami) have developed and proved successful a method of incorporating water-soluble, membrane impermeable substances into live cells using phospholipid vesicles. This was shown by measuring the uptake of 6-carboxyfluorescein and guanosine monophosphate by observing the relief of concentration quenching of fluorescence in 6-carboxy-fluorescein transferred into live retinas and by the synthesis of guanosine triphosphate from guanosine monophosphate. The fluorescence efficiency of 6-carboxyfluorescein was found to be pH dependent with a single pKa at 6.8. This was used to show the internal pH of retinal cells to be 6.8. When calcium buffers were introduced into retinal rod cytoplasm by this technique, the effect of these buffers on the retinal light sensitivity was affected as predicted by the calcium hypothesis.

These phospholipid vesicles are being used to introduce calcium sensitive dyes into retinal cells to further test the calcium hypothesis. To this effect, we have studied the properties of a new calcium sensitive azo dye, dichlorophosphonazo III. We find its $CaK_D = 10^{-6}$ M at pH 7; it is also a pH indicator. An analysis of a family of dichlorophosphonazo III absorption spectra shows this dye is capable of reporting simultaneously the activities of Mg^{2+}, Ca^{2+} and H^{+} ions. A multi-channel spectrometer was built to analyze rapid ionic changes in the retina and other thin tissues. Our tests show predicted sensitivity for these ions for transient changes in absorbency to be 10^{-4} A with a time resolution of 10^{-3} seconds.

II. The lizard retinas with pure cone cells were used to test another aspect of the calcium hypothesis. Cone outer segment membrane topology differs from that of rods; the disc space in cones is confluent with the extracellular space and thus should be more easily perturbed by changes in the cell surround than it would be for rod cells. Rapid calcium ion depletion and replenishment experiments show that the cone retina photocurrent response is quickly and transiently abolished by transient lowering of a Ca^{2+} 10^{-9} M. This result supports the hypothesis that rods and cones have similar photoexcitatory mechanism.

III. Isolated and perfused retinas can maintain its photoresponse for an extended time but cannot restore any significant amount of visual pigment following full bleaching. We (Noll and Yoshikami) have developed a method using phospholipid vesicles to restore quickly and quantitatively the visual pigment in such retinas and determined how phospholipid vesicles transfer water insoluble materials through an aqueous medium to cells. This general idea of employing phospholipids to introduce water insoluble substances into cells in an aqueous environment should be valuable in other studies, in particular for drug analysis.

We have used these phospholipid vesicles to regenerate visual pigments in live isolated retinas in order to study the biochemical pathways of regeneration and to study the photoexcitation process. For the latter we have constructed an apparatus to measure rapid photochemical kinetic and electrical transients in the retina.

In the study of visual pigment regeneration we have examined how and where retinol is 1) transported, 2) oxidized and reduced, and 3) isomerized. We have measured the solubility of retinol in water and determined its greatest upper bound to be less than 10^{-9} Molar. We find that retinol is rapidly oxidized into expoxides in aqueous solutions despite very strict precautions against oxidation. The solubility and oxidation properties of retinol indicate that its transfer between the pigment epithelium and retina is not a trivial process, especially in view of the high oxygen demand of the retinal cells. We have shown that retinol must enter the aqueous space between the retina and pigment epithelium. This was demonstrated by interposing barriers of varying porosity and thickness between the two tissues and measuring visual pigment regeneration. There is no direct cell-to-cell transfer of retinol. The migrant retinol cannot be free retinol in solution but its upper limit of packaging size must be less than 300 Å. Preliminary digestive enzyme tests show the retinol transfer packet is unaffected by free papain and immobilized trypsin and phospholipase. The transfer could involve secretion of small phospholipid or fatty acid coated oil droplets. We find the distance between the P.E. and retina to be extremely important for the well-being of the retina. An induced separation between the two tissues of only 100 μm is sufficient to prevent visual pigment regeneration. This finding has significance to the retina in cases of retinal detachment.

We have begun to investigate the biochemical pathways of retinol in the retina and pigment epithelium using phospholipid vesicles as bearers of different retinol congeners. We have discovered *inter alia* that the retinol oxidase is stereospecific for 11-*cis* retinol. This suggests the retinaldehyde reductase may be specific for the all-trans species. We are extending this study on the pigment epithelial and retinal enzyme stereospecificity and metabolism.

Significance to Biomedical Research and the Program of the Institute: Our understanding of the causes and our ability to prevent and treat numerous visual disorders depend on a clear knowledge of the processes operant in normal vision. Our finding on the importance of calcium and its control in the visual cell excitatory process and revelation of the tight coupling between photoexcitation and energy metabolism of this cell may help us to realize some of the basis for pathology in the retina. The concatenated reactions of retinol in two adjacent tissues, the pigment epithelium and retina, show these tissues are interdependent. The understanding of how retinol and other metabolites pass through the aqueous space between them has bearing on the vitality of both tissue, in particular where retinal detachment occurs.

The development of the method of introducing and measuring intercellular ion activity reporting dyes should be useful in many other areas of biomedical research. Using the phospholipid vesicle as a way to carry water insoluble

and as well as water soluble, membrane-impermeable substance into live tissues has broad ramifications which extend from pharmacology to genetic engineering and should be of general interest in all biomedical research.

Proposed Course: How the retina initiates and sustains vision are the focal points of our studies. We will continue to study the physical and chemical process involved as outlined above.

NEI Research Program: Retinal and Choroidal Diseases - Retinal Detachment/Visual Cells and Pigment Epithelium/Retinal Organization and Visual Adaptation/Special Areas of Future Interest(Low Vision/Retinal Regeneration and Transplantation)

Experimental Subject or Tissue Source: Rat/Frog/Lizard/Fish

Research Objective: Etiology

Publications:

Weinstein, S., Yoshikami, S., Henbart, P., Blumenthal, R., and Hagins, W.A.: Liposome-cell interaction: Transfer and intercelluiar release of a trapped fluorescent marker. Science 195: 489-492, 1977.

Hagins, W.A., and Yoshikami, S.: Intracellular transmission of visual excitation in vertebrate photoreceptors: Electrical effects of chelating agents introduced into rods by vesicle fusion. In Fatt, P. and Barlow, H.B. (eds.): International Symposium on Photoreception. New York, Academic Press (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00032-01 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Metaplastic Formation of Neural Retina in vitro

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Alfred J. Coulombre	Ph.D.	Head, Section on Experimental Embryology	LVR NEI
Other:	Yasuhiko Tsunematsu	Ph.D.	Postdoctoral Fellowship	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Embryology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.2	0.2	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Sheets of retinal pigmented epithelium (RPE), isolated at several developmental stages from the eyes of chick embryos, gave rise in tissue cultures to patches of differentiating neural retina. These metaplastic foci arise toward the centers of the explants. The steps identified in their appearance were: local depigmentation of the RPE sheet, first detected after three days in culture; rapid mitosis in the depigmented regions; stratification of the nuclei within these foci; and differentiation of the major layers characteristic of neural retina (NR). RPE from donors younger than developmental stage 27 gave rise to patches of NR, those from older donors did not. The polarity of metaplastic foci of NR was concordant with that of the RPE in which they arose.

Project Description:

Objectives: This project tested the feasibility of eliciting the metaplastic formation of neural retina (NR) from retinal pigmented epithelium (RPE) in tissue culture. We exploited the in vitro context, and its facilitation of experimental approaches to the nature of this metaplastic transformation, to answer the following specific questions. What is the sequence and time course of events leading to this metaplasia? Up to what embryonic age is the RPE capable of regenerating NR? What is the relationship between the polarities of the metaplastically formed NR and the RPE which generates it? Do media conditioned by NR, RPE or chondrocytes affect this type of metaplasia?

Methods Employed: Embryos of the domestic fowl were used for this study. Routine techniques of experimental embryology were supplemented with procedures drawn from histology, electron microscopy and tissue culture to study the metaplastic formation of neural retina from the RPE in vitro. A technique utilizing EDTA and Ca^{++}-Mg^{++}-free culture medium was adapted for isolation of clean sheets of RPE from embryos of different ages.

Major Findings: 1. NR forms metaplastically from the chick-embryonic RPE in vitro in a manner similar to that which had been demonstrated previously in vivo. 2. This metaplastic formation of NR occurs at random in sites toward the center of the explanted sheet of RPE. 3. The sequence of events leading up to metaplastic formation of NR are: focal depigmentation in the RPE sheet (detected after three days in culture); rapid mitosis of cells in the depigmented foci; stratification of cell nuclei in these foci; differentiation within each focus of the major layers characteristic of NR. 4. The capacity of the chick embryonic RPE to form NR metaplastically declined steadily until donor-stage 27 when it disappeared. 5. The polarity of the metaplastically formed neural retina was concordant with that of the parent RPE (outer limiting membrane of NR corresponding to RPE apical surface and ganglion-cell fiber layer corresponding to RPE basal surface). 6. The number of depigmented, preretinal foci increased until seven days of culture and thereafter remained nearly constant. 7. Deliberately co-culturing RPE and suspensions of cells from chick embryonic neural retina, demonstrated that the frequency of occurrence and the degree of maturation of foci of metaplastic formation of NR decreased as the concentration of NR cells was increased. This finding confirmed a previous study in vivo in this Section, which showed that NR inhibits the metaplastic formation of NR from RPE. It also indicates that the neural retinal foci seen in this study arose metaplastically from RPE and not from NR cells which may have been carried over inadvertently with the RPE explants. 8. Foci of metaplastic formation of lens tissue occurred in the depigmented zone of outgrowth of RPE explants but rarely toward the centers of such explants. These lentoid bodies were first detected after 10 days of culture. 9. Media conditioned by NR, RPE or chondrocytes had no effect on the metaplastic transformation of RPE into NR. By contrast, NR-conditioned medium enhanced the frequency of occurrence and the degree of maturation of lentoid bodies. 10. The metaplastic formation of NR from RPE in vitro is similar to the same process in vivo in that: it occurs only in relatively young RPE; metaplastic foci appear to arise randomly in the RPE-cell population and are probably clonally derived in both situations; the maturation of neural retina in these foci occurs more rapidly that it does

during normal development; and the polarity of the resultant NR is concordant with that of the RPE in which it arises. 11. There is also a major difference. Metaplastic formation of NR from RPE does not occur autonomously in vivo, but requires the presence of some other tissue (e.g. neural retina, otocyst); it is autonomous in vitro in the sense that it occurs in the initial absence of any tissue other than RPE. It is possible that, in vitro fetal calf serum, which is present in the culture medium, substitutes for the influence supplied in vivo by NR.

Significance to Biomedical Research and the Program of the Institute: These results contribute information on normal development and congenital abnormalities which relate to the NEI research program on Retinal and Choroidal Diseases. A feature common to a number of developmental anomalies of the eye is the duplication of the NR, especially in the region of the choroid fissure. The present study suggests that the redundant NR is produced metaplastically from the RPE. It further demonstrates that the eye is at hazard to this particular malformation only until the RPE loses its ability to give rise to NR metaplastically. It also confirms previous findings that already-differentiated NR represses the metaplastic transformation of RPE into NR. The results define further the limitations to regeneration of the NR which exist in higher vertebrates, but not in some of the lower vertebrates.

Proposed Course: This project accomplished its objectives, has been reported in an article accepted for publication and has been terminated.

NEI Research Program: Retinal and Choroidal Diseases - Developmental and Hereditary Disorders

Experimental Subject or Tissue Source: Chick

Research Objective: Etiology

Publications:

Tsunematsu, Y. and Coulombre, A.: Differentiation of neural retina in cultures of retinal pigmented epithelium of chick embryo. Devel. Biol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00178-02 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Sensitive Period in the Development of the Scleral Ossicles of the Avian Eye

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Alfred J. Coulombre Ph.D. Head, Section on Experimental Embryology LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Embryology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.0	0.0	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The scleral ossicles, a ring of membrane bones surrounding the cornea of the domestic fowl are foreshadowed in early development by transient thickenings in the overlying conjunctiva, the conjunctival papillae. Each ossicle is induced by its corresponding papilla and develops in a collagen-bearing bed deposited beneath the papilla.

L-azetidine-2-carboxylic acid (LACA) an analog of proline, was injected into the chorioallantoic veins of chick embryos to transiently disrupt collagen synthesis. Embryos treated at 5 or 6 days of incubation (but not those treated before or after this interval) developed retarded papillae and later developed ossicular rings lacking one or more ossicles.

Control embryos, injected with D-azetidine-2-carboxylic acid or with water, developed normally.

Thus, the action of LACA in aborting the induction of scleral ossicles is age-restricted and stereoisomerically-specific.

Project Description:

Objectives: A sequence of tissue interactions initiates and controls many of the morphogenetic changes involved in the normal or abnormal development of the eye. Our investigation of the nature of such interactions, their sequencing and the manner in which they exert morphogenetic control, exploits the development of the scleral ossicles, a ring of about 14 membrane bones which encircles the cornea in many submammalian vertebrates, including the domestic fowl. Each of these bones is foreshadowed in early development by a transient thickening in the overlying conjunctiva, the conjunctival papilla. The bone is induced by the papilla and develops in a collagen-bearing bed deposited beneath the papilla. This project sought to determine whether L-azetidine-2-carboxylic acid (LACA), an analog of proline which disrupts collagen synthesis, would prevent ossicular development. A further goal was to determine the period during development when the ossicular system was maximally at hazard to this teratologic agent.

Methods Employed: The study involved: 1. injection of LACA by microcatheter into the extraembryonic blood vessels of five, six, seven or nine day old chick embryos, using a method developed by this Section; and 2. analysis of the effects of this agent on the subsequent development of the conjunctival papillae and scleral ossicles.

Major Findings: 1. Injection of LACA into the chorioallantoic veins of embryos at five or six days of incubation suspended the deposition of collagen, retarded maturation of the papillae and was followed by deletion of ossicles from the bony ring. 2. Injection of LACA at five, eight or nine days of incubation did not alter the number of papillae or ossicles that subsequently developed. 3. Control embryos, injected with D-azetidine-2-carboxylic acid or with water developed normally.

Thus, the action of LACA in suspending the production of collagen and aborting the induction of scleral ossicles is age-restricted and stereoisomerically-specific. The results also call attention to the possibility that the conjunctival papilla is induced in the embryonic conjunctiva on the sixth day of incubation, a day before it normally appears grossly.

Significance to Biomedical Research and the Program of the Institute: This work aims at analyzing epithelio-mesenchymal inductive interactions in ocular development, by exploiting an optimally accessible example of this type of tissue interaction, the conjunctival papilla-scleral ossicle complex. The findings demonstrate the severely restricted developmental period during which this tissue interaction is susceptible of disruption by noxious agents.

Proposed Course: Work on this project has been completed. The project will be terminated following publication of the results.

NEI Research Program: Corneal Diseases

Experimental Subject or Tissue Source: Domestic fowl

Research Objective: Etiology

Project No. Z01 EY 00178-02 LVR

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00036-01 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development of the Chick Conjunctival Periderm and Conjunctival Papillae

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ellen Porzig	Ph.D.	Staff Fellow	LVR NEI
Other:	Alfred J. Coulombre	Ph.D.	Head, Section on Experimental Embryology	LVR NEI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Embryology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	1.0	None

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The conjunctival papillae of the chick embryo are under study to determine the roles played by the peridermal cells in the development of the underlying perilimbic sclera. Specifically, we seek to describe the timetable and regional distribution of changes in the size, shape and surface characteristics of the peridermal cells and to correlate these changes with the development of the conjunctival papillae and of the scleral ossicles which arise under the influence of the papillae.

Project Description:

Objectives: The ectoderm, which covers the surface of vertebrate embryos, is a stratified squamous epithelium comprising two layers, an inner basal layer of cuboidal cells and an outer layer of squamous cells called the periderm. While the basal layer has been shown to serve a number of important developmental functions, no functions have been demonstrated for the periderm. A favorable opportunity for identifying such functions is presented in the chick embryo by transient thickenings (papillae) in the conjunctiva. These structures appear on the seventh and eighth day of embryonic development as fourteen focal thickenings of the conjunctiva, in a ring surrounding the corneal limbus. The papillae disappear on the thirteenth day. During its brief existence, each is responsible for the induction of a bone (scleral ossicle) in the underlying mesenchyme.

This study focuses on the conjunctival periderm and seeks to answer the following questions. Are there changes in the periderm which correlate spatially and temporally with the development of the conjunctival papillae or with the pattern of development in the ring of bones induced in the underlying mesenchyme by the papillae? Specifically, do changes in the shape, size, surface characteristics or mutual attachments of the peridermal cells occur at times when or in places where the papillae are active inductively? When, during development, does the conjunctival periderm desquamate? Does desquamation occur at the same time in all regions of the periderm, or are there consistent regional differences in the time of onset of desquamation? If such regional differences exist, how do they correlate with the development and involution of the papillae?

Methods Employed: The size and shape of the conjunctival perideraml cells will be determined over a range of ages in the chick embryo in specimens stained for cell outline by a modified silver nitrate impregnation and in specimens prepared for scanning electron microscopy (SEM). Photographs and camera lucida tracings will be used for geometric and planimetric measurements of cell size and shape. SEM will be used also to follow changes in the surfaces of the cells.

Major Findings: 1. Conjunctival peridermal cells are polygonal (for the most part hexagonal) and their apposed borders form a three-rayed net. 2. Until the seventh day of incubation these cells are not elongated. On the seventh and eighth days, following the appearance of the conjunctival papillae, patches of peridermal cells become elongated along the ring formed by the papillae. Each patch of elongated peridermal cells tends to lie on one side or the other of a papilla with respect to a papilla tends to correlate with the direction in which the bone which comes to underlie it will overlap its nearest neighbors. The elongation disappears a day or two before the papillae degenerate. 3. The peridermal cells which overlie each papilla become markedly smaller than their neighbors outside the papillary zone. 4. Each conjunctival peridermal cell has sparse microvilli on its surface. Over the papillae, however, these cells develop high rugae. 5. The conjunctival peridermal cells are tightly apposed to their neighbors, except over the papillae where they partially separate from their nearest neighbors.

Significance to Biomedical Research and the Program of the Institute: Just as the embryonic corneal epithelium has been shown to dictate the three-dimensional architecture of the stromal tissue underlying it, evidence is now emerging that the embryonic conjunctiva induces tissues in the mesenchyme beneath it and determines their structure. The sharp transition between corneal stroma and the limbic sclera appears to be attributable in large measure to the activities early in development of the quite different epithelia which overlie these regions. This study uses the favorable context of the chick embryonic conjunctiva and perilimbic sclera to explore some of the developmental roles of the conjunctiva, with special emphasis on the roles possibly played by its periderm. It is hoped that these efforts will clarify some aspects of normal and abnormal shaping of the anterior segment of the eye during embryonic development.

Proposed Course: This project was initiated recently and will be continued in an attempt to reach its objectives.

NEI Research Program: Corneal Diseases - Refractive Problems and Contact Lenses

Experimental Subject or Tissue Source: Chick

Research Objective: Etiology

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00028-01 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Ribosomal RNA Synthesis in the Eastern North-American Newt, N viridescens

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: David H. Reese Ph.D. Staff Fellow LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Embryology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.1	0.1	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Ribosomal RNA (rRNA) synthesis, the initiation of which is an early major event during the transformation of iris into lens in the newt, was characterized in the TVI cell-line derived from the eastern North American newt Notophthalmus viridescens. Employing the technique of polyacrylamide gel electrophoresis, molecular-weight measurements were made on newt rRNAs using Xenopus laevis and E. coli rRNAs as standards. The molecular weights of N. viridescens 28S and 18S rRNA were found to be 1.4×10^6 and 0.7×10^6 respectively. The precursor to these RNAs had a molecular weight of 3.1×10^6. Three probable intermediates in the processing of precursor to mature rRNA were also identified. On the basis of the molecular weights of all species of RNA identified, a processing pathway, similar to that of Xenopus, has been suggested.

Some unusual features in the kinetics of precursor rRNA labelling and processing suggest the possibility that newt-cell rRNA synthesis may be controlled by the availability of essential amino acids in a manner similar to that observed in mammalian cells. A possible relationship between the availability of essential amino acids, the initiation of rRNA synthesis in the newt iris, and the control of lens regeneration is discussed.

Project Description:

Objectives: This project sought to exploit a cell line derived from an amphibian which is capable of regenerating an entire lens, in order to analyze some aspects of the synthesis of ribosomal ribonucleic acid (rRNA). In this animal, the enhanced synthesis of rRNA is an early and important event in the regeneration of a lens from the dorsal margin of the iris epithelium. Therefore, the project specifically undertook to determine the molecular weights of 28S and 18S rRNA, to identify precursors of these rRNAs and to study the kinetics of processing of the precursors.

Methods Employed: Cultures of the TVI cell line, derived from the iris of the newt, Notophthalmus viridescens were exposed to radioisotopically labelled precursors of RNA. After short-term or long-term exposure of the cultures, rRNA was extracted and subjected to polyacrilamide gel electrophoresis in the presence of labelled rRNA standards and carriers of known molecular weight, derived from E. coli and from Xenopus laevis. This permitted estimations of the molecular weights of 28S and 18S rRNAs of the TVI cell line.

Major Findings: 1. The molecular weight (M.W.) of rRNA from N. viridescens was measured as: 1.4×10^6 for 28S rRNA; and 0.7×10^6 for 18S rRNA. The M.W. of N. viridescens 18S rRNA was the same as that of a European newt (Triturus cristatus) and an anuran (Xenopus laevis). While the M.W. of 28S rRNA was the same in N. viridescens and T. cristatus it was less than that for the 28S rRNA of X. laevis.

2. Transfer RNA from N. viridescens had a M.W. of 0.026×10^6, a value comparable to that previously determined for X. laevis.

3. Short-term labelling of the rRNA of TVI cells revealed a precursor for 28S and 18S rRNA with a M.W. calculated to be 3.09 ± 0.069 (SEM) $\times 10^6$ (the range of seven determinations was $2.9\text{-}3.3 \times 10^6$). Under the conditions of culture the precursor rRNA was processed through at least three intermediates to yield 28S rRNA.

Significance to Biomedical Research and the Program of the Institute: The enhanced synthesis of rRNA in the epithelial cells of the dorsal iris is a very early event leading to the complete regeneration of a normal lens. This study characterizes the rRNAs and demonstrates the existence of precursor rRNAs. In expanding our information about early events in lens regeneration, the findings contribute to the NEI research program related to cataract.

In the course of this study the possibility was raised that the availability of essential amino acids may control the synthesis of rRNA in newt cells. This possibility is testable. It not only suggests a control mechanism for the initiation of lens regeneration, but also has wider implication for the control of the synthesis of rRNA in other tissues of the body.

Proposed Course: This study has accomplished its objectives, its results have been published and it is, accordingly, terminated.

Project No. Z01 EY 00028-01 LVR

NEI Research Program: Cataract

Experimental Subject or Tissue Source: Newt/Clawed toad/bacterium

Research Objective: Etiology

Publications:

Reese, D.: Ribosomal RNA synthesis in the eastern North-American newt, Notophthalmus viridescens. Differentiation 7: 99-106, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00177-02 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Plasma Membrane Composition and Biosynthesis in Chick Lens Fibers and Epithelia

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Peggy Zelenka Ph.D. Senior Staff Fellow LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Embryology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.25	1.25	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This project seeks to isolate and characterize the principal lipid and protein components of plasma membranes of embryonic chick lens epithelia and lens fibers, to determine whether the membrane composition changes during lens cell differentiation, and to follow any changes in the rates of synthesis or degradation of membrane components as a function of developmental time.

The phospholipid compositions of lens epithelia and lens fibers of six-day-old chick embryos have been determined, and the amount of ^{32}P incorporated into each phospholipid has been measured in vitro and in vivo. The results of these experiments indicate that the most actively metabolized phospholipids in both the lens fibers and lens epithelia are phosphatidic acid and phosphatidylinositol. Measurements of the net synthesis of each phospholipid during a two-hour labeling period in vivo indicate that the net synthesis of phosphatidic acid in the lens fibers is eight to ten times greater than in the lens epithelia, while the net synthesis of other phospholipids is two to three times greater than in the epithelia.

Project Description:

Objectives: The objectives of this project are: a) to characterize the principal lipid and protein components of plasma membranes from embryonic chick lens fibers and lens epithelial cells; b) to determine whether the differentiation of lens epithelial cells into lens fibers is accompanied by changes in membrane composition; and c) to learn whether the differentiation of lens epithelial cells into lens fibers is accompanied by changes in the rates of synthesis or degradation of components of the plasma membranes of lens cells.

Methods Employed: Lens fibers and epithelia of six-day-old chick embryos are isolated by microdissection for extraction of phospholipids. ^{32}P-labeled phospholipids are obtained either by culturing the lenses in the presence of the isotope or by injecting isotope into the embryos via the chorio-allantoic circulation. Phospholipids are separated by thin layer chromatography; radioactivity is determined either by scintillation counting or by autoradiography. Experiments in progress employ computer modeling to determine rates of synthesis and degradation of individual phospholipids.

Major Findings: The lens fibers and lens epithelia of six-day old embryonic chicks have very similar phospholipid compositions. Both are rich in phosphatidylcholine and phosphatidylethanolamine, and contain lesser amounts of phosphatidylserine, phosphatidylinositol, sphingomyelin, phosphatidic acid, phosphatidylglycerol and diphosphatidylglycerol. The fibers and epithelia differ significantly only in their sphingomyelin content. This phospholipid represents 6% of the total lens fiber phospholipid, as compared to <1% in the lens epithelia.

When the lenses of six-day-old chick embryos are labeled with ^{32}P either in vitro or in vivo, the phospholipids with the highest specific activity are phosphatidylinositol and phosphatidic acid. These phospholipids, therefore have greater metabolic activity than the other lens phospholipids.

The distribution of ^{32}P among the various phospholipids in the lens fibers differs from that observed in the lens epithelia. In particular, the proportion of label incorporated into phosphatidic acid in the lens fibers is eight to ten times greater than that in the epithelia. Since differences in phospholipid labeling may reflect metabolic changes which play a regulatory role in lens fiber differentiation, additional experiments have been undertaken to determine the rates of synthesis and degradation of the various phospholipids. For this, the specific activity of the ATP pool in the lens fibers and epithelia as well as the amount of radioactivity incorporated into each phospholipid are being determined as a function of time. Thus far, the data indicate that the net synthesis of phosphatidic acid during a two hour labeling period in vivo, is approximately nine times greater in the lens fibers than in the lens epithelia; net synthesis of all other phospholipids is two to three fold greater in the lens fibers.

Significance to Biomedical Research and the Program of the Institute: The plasma membranes of lens cells appear to play important roles in normal development and function of the lens. In addition they are centrally involved in the genesis and development of several varieties of lens cataract. Despite the widely recognized and important functions of these membranes, work on their composition, turnover, and development has begun only recently. This project focuses on changes in lens cell membranes which are associated with lens fiber differentiation. The results should have broad application in understanding normal lens differentiation and morphogenesis, and in attempts to establish etiologies for several types of cataract.

Proposed Course: This project will be continued. An attempt will be made to determine the rates of synthesis and degradation of the individual phospholipids of embryonic chick lens fibers and lens epithelia in vivo, by computer analysis of the time course of incorporation of ^{32}P into the phospholipids.

NEI Research Program: Cataract - The Normal Lens

Experimental Subject or Tissue Source: Domestic fowl

Research Objective: Etiology

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00034-01 LVR

PERIOD COVERED
October 26, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Effects of Vitamin A Deficiency on Ocular Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Louvenia Carter-Dawson	Ph.D.	Staff Fellow	LVR NEI
Other:	Toichiro Kuwabara	M.D.	Head, Section on Experimental Pathology	LVR NEI

COOPERATING UNITS (if any)

Dr. John G. Bieri	National Institute of Arthritis, Metabolism and Digestive Diseases, NIH

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Pathology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.1	1.1	0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Electroretinograms from vitamin A deficient albino rats show a decrease in amplitude of the a and b waves as early as 27 days on the diet. This decrease continues as the duration on the deficient diet lengthens. Through two months on the deficient diet, the only correlation between the structural integrity of the retina and the reduction in amplitude is that the outer two-thirds of the outer segments lose their normal staining properties. Around four months, some outer segments show signs of deterioration; however, their inner segments appear normal. Light and electron microscopic studies of the retina, pigment epithelium and their interrelationship are in progress at various levels of deficiency.

Project Description:

Objectives: In several less developed areas of the world, such as Asia and Central America, a large number of people suffer from vitamin A deficiency. This deficiency results in keratomalacia and poor vision. The early histological and cytological changes which occur in the ocular tissues have not been clearly described. This project was designed to investigate the effect of vitamin A deficiency on the maintenance of photoreceptor and pigment epithelial cell structure and functional interrelationship.

Methods Employed: Pregnant rats were fed vitamin A free diets (basic diet) one week prior to delivery and maintained on the diet through lactation. At weaning, 21 days, the basic diet of one group was supplemented with retinoic acid, a second with retinyl palmitate, and a third group received no supplement From each group electroretinograms were recorded and the retinas examined by light and electron microscopy. The animals were reared in cyclic light -- 12 hours light, 12 hours dark -- at a cage illumination 1.5-2 foot-candles.

Major Findings: Electroretinograms from the deficient albino rats showed a reduction in amplitude of the a and b waves as early as 27 days of age. The amplitude of both waves continued to decrease with duration on the diet.

Structurally, the retinas of these animals appeared normal through eight weeks. However, the outer segments in sections stained with toluidine blue showed a difference in staining intensity of the outer two-thirds. This portion of the outer segment stained considerably lighter. Whether this is related to the presence or absence of the visual pigment, rhodopsin, is unclear. Around four months, the outer half of some outer segments began to sho signs of deterioration, but the inner segments appeared normal. No loss of photoreceptor nuclei was apparent at this age.

Significance to Biomedical Research and the Program of the Institute: Results from these studies will give some further insight into the role of vitamin A in the maintenance of photoreceptor and pigment epithelial cell structural and functional interrelationship. In addition, these studies will provide information on the early histological and cytological changes which occur before signs of poor vision are manifested.

Proposed Course: Light and electron microscopic analyses of photoreceptor and pigment epithelial cell structure will be continued through late stages of vitamin A deficiency. Also, other ocular tissues, such as the corne and conjunctiva will be examined at various stages of deficiency.

NEI Research Program: Retinal and Choroidal Diseases - Visual Cells and Pigment Epithelium

Experimental Subject or Tissue Source: Rat

Research Objective: Etiology

Publications: None

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do **NOT** use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00129-05 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Anatomical and Pathological Studies of Ocular Tissues

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Toichiro Kuwabara	M.D.	Head, Section on Experimental Pathology	LVR NEI
Other:	Fujiko Huang	M.D.	Visiting Scientist	LVR NEI
	Minoru Tanaka	M.D.	Visiting Scientist	LVR NEI

COOPERATING UNITS (if any)

Simmons Lessell	M.D.	Boston University
Robert Friedlaender	M.D.	Food and Drug Administration
Shirley Wray	M.D.	Harvard University

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Pathology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.9	2.1	0.8

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Normal and pathological eye tissues of the human and experimental animals have been studied by electron microscopy in order to elucidate pathogenesis of various eye diseases.

Completed works during this fiscal year are: fine structure of the cornea, especially on the keratocyte; histopathological study on ocular changes induced by suckling mouse cataract agent; electron microscopic study of cataractous lenses of diabetic sand rats; electron microscopic and biochemical studies on experimentally induced Niemann-Pick disease-like changes in albino rats; degeneration of the pigmented ciliary epithelium by hyperosmotic shock to the monkey; fine structural studies on the conjunctiva of a case of Richner-Hanhart syndrome, and on the optic nerve and retina of a case of adrenoleukodystrophy; histologic and statistic study on metastatic skin melanoma to the eye; electron microscopic study of extraocular muscles in myotonic dystrophy.

PHS-6040

Project Description:

Objectives: The principal goal is the elucidation of the pathophysiology of disease processes, both naturally occurring and experimentally induced. Fundamental to this understanding of disease mechanisms is the greater clarification of the normal anatomy and functions of the eye.

Methods Employed: Eye tissues of the human and various experimental animals were fixed in glutaraldehyde and examined by transmission and scanning electron microscopy. Individual experimental procedures will be described under the headings of major findings.

- A large number of clinicopathological specimens which were submitted to this laboratory from the NEI Clinical Branch and many other eye research centers of the nation were studied for specific aims by electron microscopy and histochemistry.

Major Findings:

Studies on the cornea

Fine structure of the cornea

Although fine structural investigations on each part of the cornea have been reported by several authors, a comprehensive discussion on the structure of the cornea as a whole has not been available since 1969. A large amount of data on cytologic studies of the cornea of the human and experimental animals was re-evaluated. With additional findings, a summary report on the corneal structure was made.

This study has demonstrated that the keratocyte is capable of movement as a whole and/or in processes, sensitively responding to various stimulations. Phagocytic activity of exogeneous particles is unexpectedly small. Phagocytosis in the stroma is found to be taken care of mainly by histiocytes and leucocytes which are abundantly present within the normal stroma. Another worthwhile finding is that there are tissue spaces along keratocytes which are connected to each other with their processes. The space may serve as the intracorneal channel.

Studies on the lens

SMCA-induced cataract

The suckling mouse cataract agent (SMCA), a member of spiroplasma, was inoculated into postnatally-developing mouse brains. These animals had a high incidence of cataract formation. Pathologic changes in the lens included: proliferation and posterior extension of lens epithelium; increased accumulation of capsule material and formation of lens fibers at abnormal locations. The histological changes of this experimental cataract are similar to those of congenital cataracts which are induced by certain fetal viral infections.

Cataractous lenses of diabetic Egyptian sand rats

A diabetes mellitus-like syndrome was induced in the Egyptian sand rat (Psammomys obesus) by changing its diet from leafy vegetables to laboratory chow. Cataractous changes developed in all experimental animals after about three weeks feeding of the high caloric diet. The first lenticular change was marked swelling and then degeneration of the cortical cells. The surviving lens cells proliferated and formed islands in the subcapsular region. Nodular masses of the fibrous tissue were formed around the proliferating cells. These changes are similar to those of cataracts in diabetes mellitus of the human.

Senile cataract

A great number of lenses with senile cataract had been sent to this laboratory for cytological studies from members of the National Eye Institute Cooperative Cataract Research Group. Lenses were studied grossly and photographed and examined by histology and scanning and transmission electron microscopy. Flat preparations and histological sections revealed that the epithelial cells became progressively sparse with age. Several acellular foci were formed. Electron microscopy of the remaining cells showed that the cytoplasm had lost the normal structure. The cataractous changes of the anterior cortex were regularly localized beneath the abnormal epithelium. These findings indicated that cytological changes in the epithelium proceeded the occurrence of the senile changes in the lens fibers.

Lentoid body

In collaboration with Dr. Paul Russell of LVR, lentoid bodies of humans and normal and cataractous mice were studied by scanning and transmission electron microscopy. Cells of the lentoid bodies are loosely packed and produce basal lamina substances between them. Some cells begin to form crystalline protein granules. Electron microscopic demonstration of ATPase in these cells is underway.

Experimental Niemann-Pick disease

Following intraperitoneal injections of AY 9944, a cholesterol synthetase inhibitor, into postnatally developing rats (50 mg per kg body weight) abundant inclusion bodies were produced in ganglion, glia, amacrine and horizontal cells of the retina, glia cells of the optic nerve, pigment epithelial cells of the retina and ciliary body, and lens fibers. Electron microscopic and biochemical studies revealed that characteristics of the inclusion body were identical to those of Niemann-Pick disease of the human. The experimentally induced inclusion bodies were different from nonspecific debris of degenerative cells and the inclusion-laden cells were otherwise cytologically normal. This experiment suggested that inhibition of cholesterol synthesis involves the pathogenesis of this hereditary disease.

Glaucoma study

Effect of hyperosmotic agents on the ciliary epithelium

2M urea or 2M DL-lactamide were perfused into the internal carotid artery of rhesus monkeys. Both layers of the ciliary epithelium were severely damaged by this hyperosmotic shock. The non-pigmented epithelium recovered to an almost normal structure within a short period of time, but the pigment epithelium especially of the pars plana became degenerative. The intraocular pressure of the perfused monkey was extremely low for about one month, but gradually came back to a near normal value. However, the damaged pigmented epithelium did not recover. The number of giant vacuoles in the endothelium of Schlemm's canal decreased while the intraocular pressure was low, but increased gradually with the recovery of the pressue. This experiment suggested a possibility of the presence of several other factors in regulating aqueous humor production besides the generally understood mechanism.

Essential iris atrophy

A case of advanced essential iris atrophy was studied by electron microscopy. The result was reported with Drs. Kaiser-Kupfer and Kupfer. The study showed that the involved iris stroma was markedly atrophic, but the dilator muscle layer was strikingly well-preserved. In some areas, proliferation of the dilator muscle was indicated. This caharacteristic histopathologic change may be related to the occurrence of frequently accompanied glaucoma.

Clinicopathological study

Adrenoleukodystrophy

Adrenoleukodystrophy is an x-chromosome-linked recessive disease characterized by primary atrophy of the adrenal gland, degeneration of white matter of the central nervous system, and blindness. Eyes of a ten-year-old boy with this disease were sent to this laboratory from Harvard University for special studies. Histological and electron microscopical studies revealed marked demyelination of the optic nerve and loss of retinal ganglion cells.

Richner-Hanhart syndrome

Richner-Hanhart syndrome with tyrosinemia was recognized in a mentally retarded adolescent boy. The conjunctiva biopsies were sent to this laboratory from Harvard University for special studies. Electron microscopic study revealed that the conjunctival epithelial cells, subepithelial fibrocytes and blood vessel endothelial cells accumulate large inclusion bodies which contain tyrosine-like fine crystals. It was hypothesized that cells are in the process of removing excess tyrosine from the blood stream and the tissue fluid.

Malignant melanoma of the skin: metastasis to the eye

Histopathologic examination of the eyes of 15 consecutive patients with metastatic malignant melanoma arising in the skin showed evidence of intraocular

metastasis in five patients. The metastases were microscopic, epithelioid, minimally pigmented, and occurred in both the choroid and the retina. All patients were asymptomatic. Those patients with ocular metastases had primary malignant neoplasms of the superficial, spreading variety and associated central nervous system metastases.

Myotonic dystrophy

Extraocular muscles of two patients with ophthalmoplegia secondary to myotonic dystrophy were sent to this laboratory from Boston University. Electron microscopic study revealed that the main change was disorganization in the arrangement of myofibrils rather than degeneration of the cells. Diseased muscle cells contained randomly distributed, short and irregular myofibrils and individual myofilaments. The cytologic appearance of these muscle cells was similar to that of developing muscle cells. The pathogenesis of the myopathy in myotonic dystrophy may be related to myofibrillogenesis and its maintenance.

Anatomical studies on normal eye

A newly revised chapter of the eye for the textbook Greep's Histology edited by Leon Weiss has been submitted for printing. New concepts on the fine structure of the eye have been emphasized in this chapter. Embryological development of various parts of the eye, especially anterior portions, has been extensively studied electron microscopically.

Significance to Biomedical Research and the Program of the Institute: There are few laboratories in this country which are capable of pursuing these disease-related basic research studies on the eye tissues. This laboratory is one of them. Significant meanings in understanding the pathogenesis of various diseases of the eye have been obtained through the results obtained in the present investigations.

Proposed Course: Similar projects are actively ongoing and will be continued in the next fiscal year.

NEI Research Program: Retinal and Choroidal Diseases - Developmental and Hereditary Disorders/Diabetic Retinopathy and Other Vascular and Circulatory Abnormalites/Tumors/Inflammatory Disorders/Uveal Tract; Corneal Diseases - Corneal Edema, Dystrophies, and Inherited Disorders/Corneal Transplantation and Stromal Injury and Repair/Tumors and Other Lid, Conjunctival, and Orbital Problems; Cataract - The Normal Lens/Diabetic Cataract/Cataract Induced by Drugs, Radiation, and Secondary to Other Eye Disorders; Glaucoma - Etiology of Glaucoma (Primary Glaucoma--Open-Angle Glaucoma/Secondary Glaucomas)

Experimental Subject or Tissue Source: Rhesus monkey/Rat/Mouse/Human

Research Objective: Etiology, Diagnosis, Treatment

Publications:

Kuwabara, T.: The corneal stroma cell. In Yamada, E. and Mishima, S. (eds.): The Structure of the Eye III, Jap. J. Ophthalmol. 1976, 39-47.

Kuwabara, T.: Current concepts in anatomy ahd histology of the cornea. In King, J.H. and McTigue, J.W. (eds.): The II World Congress on the Cornea. Washington, D.C., 1976, Butterworths (in press).

Friedlaender, R.P., Barile, M.F. and Kuwabara, T.: Ocular pathology induced by the suckling mouse cataract agent (SMCA). Invest. Ophthalmol. 15: 640-647, 1976.

Kuwabara, T. and Okisaka, S.: Electron microscopic study of cataractous lenses of diabetic sand rats (Psammomys obesus). Doc. Ophthalmologica Proceedings - Progress of Lens Biochemistry Research, 1976, 7-15.

Russell, P., Fukui, H.N., Tsunematsu, T., Huang, F.L. and Kinoshita, J.H.: Tissue culture of lens epithelial cells from normal and Nakano mice. Invest. Ophthalmol. 16: 243-246, 1977.

Sakuragawa, M.: Niemann-Pick disease-like inclusions caused by a hypocholesteremic agent. Invest. Ophthalmol. 15: 1022-1027, 1976.

Sakuragawa, N., Sakuragawa, M., Kuwabara, T., Pentchev, P.G., Barranger, J.A. and Brady, R.O.: Niemann-Pick disease experimental model: Sphingomyelinase reduction induced by AY-9944. Science 196: 317-319, 1977.

Okisaka, S., Kuwabara, T. and Rapoport, S.I.: Effect of hyperosmotic agents on the ciliary epithelium and trabecular meshwork. Invest. Ophthalmol. 15: 617-625, 1976.

Kaiser-Kupfer, M., Kuwabara, T. and Kupfer, C.: Progressive bilateral essential iris atrophy. Am. J. Ophthalmol. 83: 340-346, 1977.

Bienfang, D.C., Kuwabara, T. and Pueschel, S.M.: The Richner-Hanhart syndrome, Report of a case with associated tyrosinemia. Arch. Ophthalmol. 94: 1133,-1137, 1976.

Wray, S.H., Cogan, D.G., Kuwabara, T. and Schaumburg, H.H.: Adrenoleukodystrophy with disease of the eye and optic nerve. Am. J. Ophthalmol. 82: 480-485, 1976.

Fishman, M.L., Tomazewski, M.M. and Kuwabara, T.: Malignant melanoma of the skin metastatic to the eye: Frequency in an autopsy series. Arch. Ophthalmol. 94: 1309-1311, 1976.

Kuwabara, T. and Lessell, S.: Electron microscopic study of extraocular muscles in myotonic dystrophy. Am. J. Ophthalmol. 82: 303-309, 1976.

Kuwabara, T.: Species difference of the pigment epithelium. In Zinn, K.M. and Marmor, M.F. (eds.): The Retinal Pigment Epithelium. Cambridge, Harvard Press (in press).

Kuwabara, T. and Cogan, D.G.: The eye. In Weiss, L. and Greep, R.O. (eds.): Histology, 4th edition. McGraw-Hill, New York, 1977, p. 1119-1164.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00131-05 LVR

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Light Effect on the Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Toichiro Kuwabara	M.D.	Head, Section on Experimental Pathology	LVR NEI
Other:	W. Gerald Robison, Jr.	Ph.D.	Geneticist/Cell Biologist	LVR NEI
	Masakazu Funahashi	M.D.	Visiting Scientist	LVR NEI
	Atsushi Mizukawa	M.D.	Visiting Scientist	LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Experimental Pathology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.9	1.7	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Effect of visible light to the retina has been studied by electron microscopy. The following results have been obtained: 1) Phagocytic activity of the pigment epithelium against latex spheres is influenced by the size of the sphere. 2) Phagocytic activity of the pigment epithelium following light damage of the retina has been studied by exposing rats to fluorescent lamps at 150 foot-candles. Phagocytic activity is first accelerated by light, but eventually is diminished when the light damage becomes severe. Also, the phagocytic activity is controlled by the circadian rhythm. 3) Synaptic organs of the photoreceptor cells show marked cytologic changes by light exposure. One of the early changes is proliferation of the paramitochondrial membranes. The cone cells are resistant to light exposure. 4) Photoreceptor cells of the developing rat continue outer segment production under bright light (400 foot-candles) for more than two weeks without showing severe damage. Further exposure causes severe damage in the newly-formed outer segments.

Project Description:

Objectives: This project has been selected to help elucidate the pathogenesis of some degenerating diseases of the human retina.

Methods Employed: Albino rats were placed in uniformly illuminated cages for various time periods. The light source was cool fluorescent light and exposure was given by day and night (12-12 hour) cycles. Retinas of the exposed animals were studied by electron microscopy.

Major Findings:

Phagocytosis of the pigment epithelium

Latex spheres

Polystyrene spheres in various sizes were injected into the subretinal space of rhesus monkeys. The pigment epithelium and retina were studied by electron microscopy. In the first few hours, the pigment epithelial cells actively phagocytized fragments of the outer segment produced by the injection injury. The spheres were engulfed at a considerably later stage. By the sixth hour, the larger spheres (1.0 μm) which had been surrounded by the microvilli were taken into the cell. Phagocytized spheres were usually distributed individually within the cytoplasm. On the other hand, the smaller (0.1 μm) spheres aggregated into clusters of 10-20 and were coated by a mucopolysaccharide substance before they were engulfed by the pigment epithelium within 24 hours. When a mixture of 0.1 and 1.0 μm spheres was injected, the cell handled the two kinds of spheres separately, evoking the pattern as described above. The size of the particles appears to be a determining factor in phagocytic activation.

Circadian cycle

Normal albino rats that had been kept under the dimmed cyclic light (two-five foot-candles for 12 hours and total darkness for 12 hours) showed an active phagocytosis of shed outer segments in the pigment epithelium at the beginning of the light period, but no activity during the dark period. When the same animals were exposed to the same cycles, but with 150 foot-candles, the phagocytic activity was profoundly accelerated during the similar time period. After the exposure of five cycles or more, the retinal outer segments were severely damaged and the phagocytic activity of the pigment epithelium diminished.

Light effect on the synapsis of the photoreceptor cell

The photoreceptor synapses of albino rats show considerable pathologic changes following fluorescent light exposure. The changes in the synapses and in the lamellar membranes of the outer segments progress simultaneously.

Membranes proliferated in the paramitochondrial zone of the rod synaptic spherule and fine budding of the smooth endoplasmic reticulum in the cone

pedicle occurs within one hour's exposure to the brightness of 500 foot-candles. Proliferating paramitochondrial membranes have no cytochrome c oxidase activity and degenerate together with mitochondria after further exposure. The cone pedicles remain relatively intact in the photically damaged retina.

Light effect on the developing retina

The effect of bright light on the developing albino rat was studied electron microscopically. The newly-formed outer segment lamellar membranes of newborn rats, raised in continuous bright light appeared to be less sensitive to the damaging effects of light, compared to rats raised under normal light conditions for two weeks. It seemed to take about two days before the newly-formed membranes showed photo damage after continuous exposure to fluorescent lamps. The same brightness damaged the adult outer segments within a few hours. Despite the severe damage to the outer segments, the rest of the retina developed normally for one month, and then the photoreceptor cell bodies degenerated. The retinas which had been exposed for two weeks since birth showed considerable damage, but these retinas regenerated in six months.

Significance to Biomedical Research and the Program of the Institute: Results obtained from the present experiments are directly useful in further understanding of the retina - pigment epithelium interrelationship. Elucidation of the basic mechanism of the pigment epithelium related to light exposure is the first step in understanding of the retinal diseases of the human.

Proposed Course: Similar studies will be continued. Effects of cyclic light exposures to the retina is now under intense investigation.

NEI Research Program: Retinal and Choroidal Diseases - Visual Cells and Pigment Epithelium/Special Areas of Future Interest(Toxic and Environmental Disorders

Experimental Subject or Tissue Source: Rhesus monkey/Rat/Mouse

Research Objective: Etiology

Publications:

Funahashi, M., Okisaka, S. and Kuwabara, T.: Phagocytosis by the monkey pigment epithelium. Exp. Eye Res. 23: 217-225, 1976.

Kuwabara, T. and Funahashi, M.: Light damage in the developing rat retina. Arch. Ophthalmol. 94: 1369-1374, 1976.

Kuwabara, T.: Photo-thermal effects on the pigment epithelium. In Zinn, K.M. and Marmor, M.F. (eds.): The Retinal Pigment Epithelium. Cambridge, Harvard Press (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00149-04 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)
Ultrastructure and Function of the Pigment Cells of the Eye

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	W. Gerald Robison, Jr.	Ph.D.	Geneticist/Cell Biologist	LVR NEI
Other:	Toichiro Kuwabara	M.D.	Head, Section on Experimental Pathology	LVR NEI
	Gerald Chader	Ph.D.	Head, Section on Retinal and Corneal Metabolism	LVR NEI

COOPERATING UNITS (if any)
None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Experimental Pathology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
2.1	1.1	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The ability of the retinal pigment epithelium to store vitamin A was tested by injecting mice with retinol acetate or retinol palmitate and examining their retinas and livers using fluorescence and electron microscopy. The results showed clear, dose-related increases in the numbers and sizes of vitamin-A-storing lipid droplets in the stellate cells of the liver. Concomitantly, more conservative increases in similar lipid droplets occurred in the pigment epithelium but not in other cells of the retina. Such lipid droplets may represent physiological sites of vitamin A storage which are important for the maintenance of photoreceptor cells by the retinal pigment epithelium.

Electron microscopic histochemistry for catalase demonstrated that peroxisomes, like the putative vitamin-A-storing lipid droplets were distributed along the basal and lateral cell surfaces of the pigment epithelium where receptors for plasma retinol-binding protein have been reported. Peroxisomes may play a role in the reactions related to the esterification and sequestering of vitamin A.

Project Description:

Objectives: Study the structural and functional interrelationships that exist between the pigment epithelium and the visual cells of the eye. We propose to examine how the pigment epithelial cells are involved in the maintenance of photoreceptor cells and what specific functions are lacking in various experimental and pathological cases.

Methods Employed: Hypervitaminosis A was induced by intramuscular injections of retinol acetate or retinol palmitate. In order to locate the sites of vitamin A storage, fluorescence microscopy was used to detect vitamin A-specific fluorescence, and then electron microscopy, combined with light microscopy of one micron sections, was performed. Mainly the retina and liver were studied.

Ultrastructural histochemistry for demonstration of catalase was used to study the distribution of peroxisomes in relation to the vitamin-A-storing lipid droplets. Littermates of inbred C57BL/6J mice were used to minimize variables.

Major Findings: Lipid droplets of the pigment epithelium increased in number depending on the availability of vitamin A, suggesting that they serve as physiological storage sites of this important component of photoreceptor cells. No changes in lipids of the retina or liver were observed in mice injected with retinoic acid or with peanut oil alone. Peroxisomes which contain catalase and often are involved in the transport, storage, and turnover of lipids were closely associated with the putative vitamin-A-storing lipid droplets along the basal and lateral cell surfaces. Since these cell surfaces were reported to have receptors for plasma retinol-binding protein, peroxisomes may be involved in the reactions related to the esterification and storage of vitamin A in the pigment epithelium.

Significance to Biomedical Research and the Program of the Institute: A healthy retina depends on a functional pigment epithelium, yet little is known about the specific functions and requirements of pigment epithelial cells. The visual process involves dynamic exchange of vitamin A between the photoreceptor cells and the pigment epithelium. The identification of vitamin A stores within pigment epithelial cells provides a visible criterion for assessing the functional state of these important cells in health and disease. An elucidation of the precise relation of peroxisomes to vitamin-A-storing droplets should be very significant.

Proposed Course: It is believed that lipofuscin granules contain retinoyl complexes deriving from retinoic acid. Their relation to phagocytosis, intracellular digestion, vitamin A turnover, and other factors need to be investigated. Lipofuscin granules in the retinal pigment epithelium and ciliary epithelium in various mouse mutants of different ages under experimental treatments with light and vitamin A will be studied.

NEI Research Program: Retinal and Choroidal Diseases - Visual Cells and Pigment Epithelium

Project No. Z01 EY 00149-04 LVR

Experimental Subject or Tissue Source: Mouse

Research Objective: Etiology

Publications:

Enriques, N., Israel, P., Bergsma, D., Robison, W.G., Jr., Whikehart, D. and Chader, G.: Neural retinal and pigment epithelial cells in culture: Patterns of differentiation and effects of prostaglandins and cyclic AMP on pigmentation. Exp. Eye Res. 22: 559-568, 1976.

Robison, W.G., Jr. and Kuwabara, T.: Light-induced alterations of retinal pigment epithelium in black, albino, and beige mice. Exp. Eye Res. 22: 549-557, 1976.

Robison, W.G., Jr. and Kuwabara, T.: Vitamin A storage and peroxisomes in retinal pigment epithelium and liver. Invest. Ophthalmol. (in press).

Robison, W.G., Jr. and Kuwabara, T.: Albino-beige mouse: Lysosomal dysfunction in retinal pigment epithelium. Invest. Ophthalmol. (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00026-06 LVR

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Physiology of the Primate Visual System

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Peter Gouras	M.D.	Head, Section on Neurophysiology	LVR NEI
	Francisco deMonasterio	M.D., D.Sc.	Visiting Scientist	LVR NEI
	Eberhart Zrenner	M.D.	Fogarty Fellow	
Other:	Avery Dickinson Nelson	Ph.D.	Senior Staff Fellow	LVR NEI

COOPERATING UNITS (if any)

Max-Planck Institute, Bad Nauheim, FRG

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Neurophysiology

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.4	1.4	0.0

CHECK APPROPRIATE BOX(ES)

☒ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

It is the long range objective of this project to study the neural organization underlying visual perception in primates. The topics of present interest are: i) chromatic, temporal and spatial properties of neurones of the retina, lateral geniculate nucleus, primary visual cortex and extra-striate cortex, ii) how this information is processed along these center, and iii) how these single cell data are related to psychophysical results in humans.

Project Description:

Objectives: To study the neural organization underlying visual perception in primates.

Methods Employed: Electrophysiological recordings from single neurons in the retina and visual cortex of anesthetized, paralyzed macaque monkeys; correlation of the distribution of single cell varieties and morphological cell types as seen by electron and light microscopy; the use of refined optical stimuli to define quantitatively spatial, temporal and chromatic properties of these neurons.

- Major Findings: The results can be grouped into three broad areas. The first involves a completion of work begun by P. Gouras in Freiburg in collaboration with J. Kruger on single cells in visual cortex. The second involves his collaborative project here on single cells studies of the retina being done with E. Zrenner, a visiting fellow from the Max Planck Institute in Bad Nauheim. The third involves intracellular studies of cells in primate retina being done together with F. de Monasterio.

The first project on visual cortex is being brought to completion and being prepared for publication, since the results are still only available in abstract form. These experiments were designed to determine the responsiveness of single cells or cell groups in foveal striate and prestriate cortex (areas 17, 18 and V4) to four colors (red, yellow, green and blue) presented as slowly moving slits or squares, quasi-simultaneously and over a large number of trials in order to minimize the effects of uncontrolled changes in neuronal excitability. This technique revealed that the color selectivity of most neurons depends upon the slit length. As length increases the effectiveness of red and blue increases relative to that of yellow and green lights. The effect is immediate and not due to response saturation. It can only be explained by assuming that signals from the most common center-surround color-opponent geniculate cells reach all of these cortical cells. This result requires reconsideration of the so called hypercomplex cell classification in monkey visual cortex since such cells must now be considered color dependent.

Color selectivity varies significantly from cell to cell ranging from a small group which responds exclusively or almost exclusively, to one color to the majority which respond to all colors to some degree in what seems to be a continuum. This variation is greater in tangential than in radial directions through visual cortex, which implies the existence of cortical slabs or columns of color selectivity.

The overall pattern of color selectivity varies quantitatively but not qualitatively among areas 17, 18 and V4 of visual cortex. Receptive fields of cells are large in prestriate cortex (areas 18 and V4) and color selectivity seems to be preferred in area V4, but otherwise each of these three areas have similar proportions of color selective cells and therefore must presumably process information about color in similar ways. What seems to be apparent is that color information is widely distributed among cells in visual

cortex and is being used to a great extent to facilitate contour detection as well to perceive color, per se.

The second project has been in collaboration with E. Zrenner and has been concerned with obtaining a better understanding of the blue sensitive cone mechanism. Our approach has been to study the responses of single ganglion cells subserving the foveolar, foveal and perifoveal retina of the rhesus monkey. In certain cells the blue-sensitive cone mechanism can be completely isolated and studied separately from the other two cone mechanisms. Several important properties of this receptor mechanism differ significantly from those of the red and green-sensitive cone mechanisms; these differences involved spatio-temporal resolution, receptor density, and center-surround polarity. Understanding the role of the blue cones in primate vision is crucial for understanding the neuronal organization of color and form perception in visual cortex.

The third project is in collaboration with F.M. de Monasterio has been directed to intracellular recordings from cells (especially horizontal cells) in perfused rhesus monkey retina. S-potentials have been obtained in this system, which we are tentatively classifying as horizontal cells pending dye injection identification. These responses suggest that both rods and cones contribute signals to the same horizontal cell (paralleling results obtained in perfused cat and rabbit retina). F.M. de Monasterio and also E. Zrenner have been able to detect these horizontal cell responses in the intact monkey retina so that we feel confident that we shall be able to begin to understand something about cone interactions in the inner nuclear layer of primate retina in the near future.

Significance to Biomedical Research and the Program of the Institute: Understanding the cellular organization of rhesus monkey vision is extremely valuable for understanding human vision, which at present can only be studied by indirect (usually psychophysical) methods. This is especially important for a small region of the primate retina, the fovea, which provides an enormous and dominant input to visual cerebral cortex and when destroyed, in man, is tantamount to blindness, legally. Understanding the neural circuitry of this foveal mechanism from retina to visual cortex will provide us with a basic scheme for appreciating old and developing new techniques to study visual function in normal and diseased human subjects.

Proposed Course: To continue the current experiments and to utilize the anatomical expertise of A.D. Nelson in correlating function with microstructure in primate retina, similar to what we have been doing in cat retina.

NEI Research Program: Retinal and Choroidal Diseases - Retinal Organization and Visual Adaptation

Experimental Subject or Tissue Source: Monkey

Research Objective: Etiology

Publications:

de Monasterio, F.M., Gouras, P., and Tolhurst, D.J.: Spatial summation, response pattern and conduction velocity of ganglion cells of the rhesus monkey retina. Vision Res. 16: 674-678, 1976.

Kruger, J., and Gouras, P.: Many cells in visual cortex use wavelength to detect borders and convey information about color. Exp. Brain Res. Suppl. 1: 407-411, 1976.

de Monasterio, F.M., and Gouras, P.: Responses of macaque ganglion cells to far violet lights. Vision Res. (in press).

Zrenner, E.: Color opponency in visually evoked cortical potentials (VECP) in man. ARVO Abstracts, p. 157, suppl. to Invest. Ophthalmol., 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00005-05 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Electrophysiological Studies of Mammalian Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Ralph Nelson	Ph.D.	Staff Fellow	LVR NEI
	Francisco M. deMonasterio	M.D., D.Sc.	Visiting Scientist	LVR NEI
	Peter Gouras	M.D.	Head, Section on Neurophysiology	LVR NEI
	Eberhart Zrenner	M.D.	Fogarty Fellow	LVR NEI
Other:	Edward V. Famiglietti,Jr.	M.D., Ph.D.	Guest Worker	

COOPERATING UNITS (if any)

Helga Kolb, Ph.D., Laboratory of Neurophysiology, NINCDS, NIH, Bethesda, Md. 20014; Max-Planck Institute, D-635 Bad Nauheim, FRG

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Neurophysiology

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.5	3.5	0.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The goal of this project is to obtain information about the physiological properties and anatomical interrelationships of neurons in the mammalian retina. The aspects of retinal physiology and anatomy with which we have dealt in the past annum are: the spectral classes of photoreceptors in cats and rabbits and the chromatic interactions among the cones; the morphological basis of the ON center and OFF center pathways of the cat retina, and a comparison of the response properties of single units in the parallel rod and cone pathways of both cats and rabbits; and finally, the pharmacological role of the cyclic nucleotides in the responses of mammalian rods. At the single unit level, electrophysiological recordings have been made, both intracellularly and extracellularly, and single units have been intracellularly injected with a variety of stains. Anatomically the synaptic interrelationships of single units have been studied electromicroscopically in serial ultrathin sections, and in the light microscope such single unit reconstructions have been compared to similar units stained with the Golgi technique, or to units electrophysiologically studied and stained. At the multiunit level the massed responses of retinal neurons as reflected in the electroretinogram have been studied.

Project Description:

Objectives: To understand the functional organization of mammalian retinas and its relationship to disease states.

Methods Employed: Intracellular and extracellular recording of neural responses to light from the in vitro, arterially perfused eyecups of cat, rabbit and, in a limited number of experiments, macaque; definition of rod-cone and cone-cone interactions through the use of action spectra, or intensity response functions to matched monochromatic stimuli in the presence of neutral or selective chromatic backgrounds; receptive field mapping; intracelluar staining of retinal neurons with Procion and other dyes; histology and fluorescence microscopy; comparison with Golgi stained and electronmicroscopically studied neurons in these retinas.

Major Findings:

ON and OFF center ganglion cells in the retina of the cat

Ganglion cells in the retina of the cat were stained by intracellular dye injection after recording their responses to photic stimulation. All cells encountered were divided into those giving ON responses and those producing OFF responses, and the level of dendritic branching of these two groups was compared. Cells giving OFF responses were found to branch high in the inner plexiform layer, near the amacrine cell bodies (sublamina a); while those giving ON responses were found to branch lower in the inner plexiform layer (sublamina b). The inner plexiform layer was found to contain a single stratum of capillaries which conveniently divides sublamina a from sublamina b, so that, of 21 stained ganglion cells there were 10 ON center units, all of which branched below the capillary bed (in sublamina b), in addition, there were 10 OFF center units, all of which branched above the capillary bed (in sublamina a). One unit was physiologically ON-OFF in its response properties; the plane of the capillary bed bisected the broad dendritic stratification of this unit. Thus whether a ganglion cell is ON center or OFF center can be unambiguously determined from the level of branching of its dendrites and the notion that ON-center and OFF center ganglion cells branch at random levels in the inner plexiform layer must be rejected at the 1 in 2^{20}th level (one in a million).

Dye-injected cells have been compared to ganglion cells impregnated by Golgi methods, and have been identified as belonging to one of three morphological classes based on cell size. Cells giving ON responses and cells giving OFF responses were found in each of these three classes, and the sign of the response only correlated with the level of dendritic branching. Thus no other morphological feature of a cell except stratification appears to be important in determining the sign of its center response.

The rod and cone inputs to some cells were characterized by comparing their responses to deep red, and blue rod-matched stimuli over a two log unit range starting at dark-adapted threshold. About half the cells appeared to be rod dominated under these conditions, whereas the other appeared to have mixed rod and cone signals. The receptive fields of some cells were measured

by moving a long narrow slit stimulus incrementally across the surface of the retina. Under these conditions receptive field centers could be either somewhat smaller or as much as six times larger than the dendritic field of the cells. This finding suggests that the often-held assumption that dendritic fields of ganglion cells ought to match receptive fields, or at least bear a linear relation to the latter, must be viewed with some caution. The stratification of ganglion cells into ON and OFF layers parallels the distribution of the axonal terminals of the flat and invaginating cone bipolars. Flat cone bipolars are in a position to contact OFF center ganglion cells (in sublamina a) and invaginating cone bipolars are in a position of contact ON center ganglion cells (in sublamina b). In the electron microscope just such connections have been observed between the ribbon synapses of the cone bipolar cells and ganglion cell dendrites. Remarkably the OFF center ganglion cells, whose dendrites must traverse the sublamina of the invaginating cone bipolar axon terminals en route to sublamina a never make a single connection with their terminals, restricting their connections in sublamina b to amacrine cells.

Chromatic interactions in the rabbit retina

The responses of neurons in the rabbit have been explored using intracellular and extracellular electrodes and dye staining techniques. With few exceptions previous studies of the rabbit retina have suggested that this species has only two morphologically and functionally different types of receptor: rods and 'green' cones. Electroretinographic studies in the rabbit's eyecup show that this retina contains, in addition to rods and green cones, blue-sensitive cones. Spectral sensitivity measurements show that these receptors absorb maximally at about 430 nm, 500 nm and 520 nm. Mass b-wave responses have a spectral sensitivity suggestive of antagonistic interactions between signals from blue and green cones. This has not been seen in action spectra based on a-wave or PIII component responses suggesting spectral interactions might exist at the level of inner nuclear layer cells, but possibly not at the level of photoreceptors. Among the ganglion cells, a fraction shows color-opponent responses. Two main types were found. In one, on-depolarizing responses and on-hyperpolarizing responses receive input from both blue and green cones (e.g. BG/G, BG/B). Two types of horizontal cells have been found, both receiving mixed green cone and rod input; one type is cone-dominated while the other is rod-dominated, resembling similar cell types described in the retina of the cat. Neither horizontal cell type shows an obvious blue cone input, either depolarizing or hyperpolarizing, with intense selective chromatic adaptation green cones and rods. The results suggest that although input from blue cones contributes to spectral interactions at the ganglion cells and, possibly, inner nuclear layer cells, it does not seem to contribute to such interactions at the photoreceptor-horizontal cell level. Assuming that in the rabbit there is a negative feedback between receptors and horizontal cells, the above results suggest that the feedback between each cone type and the corresponding horizontal cell is either private (the spectral sensitivity of the latter would depend only on the absorption of the corresponding cone type) or spectrally asymmetric (green cones would feedback onto blue ones, but blue cones would not feedback onto green cones).

Spectral opponency in the cat electroretinogram

Spectral opponency and asymmetry between cone mechanisms have been more thoroughly studied in the cat electroretinogram. In the d.c.-ERG of the isolated perfused cat eye two cone mechanisms (λmax=450 and 555 nm) can be identified by their action spectra obtained by constant response criteria with monochromatic Ganzfeld-stimuli in the presence of intense chromatic adaptation (12 experiments).

At the onset of the light stimulus and in the presence of strong yellow adaptation both cone mechanisms appear to sum their contributions to the negative on-response (P III) producing a broad, flat, single-peaked action spectrum, fitting a summation of 450 and 555 nm Dartnall-nomograms when corrected for lens absorption. Under the same conditions they appear to oppose each other in their contribution to the positive on-response (b-wave) producing a double peaked action spectrum (peaks near 450 and 555 nm) with a large sensitivity loss near 500 nm, fitting a subtraction of both nanograms. This double-peaked function cannot be due to interaction between a 555 nm cone mechanism and rods because the threshold sensitivity of the short wavelength branch remains unaltered over a 16-fold increase in yellow adaptation whereas that of the long wavelength branch follows the Weber-Fechner law.

When isolated, the sigmoidal intensity-response function for the 450nm-mechanism has a lower slope and saturates at several V maximum amplitude; that of the 555nm-mechanism is steeper and cannot be saturated with our maximum intensity. The intensity response function for the former could be made to approximate the latter by multiplying it by a factor of at least 10, possibly the ratio of 555 to 450nm cones.

At the offset of the light, both cone mechanisms generate a slow negative response but only the 555nm-mechanism produces a quick positive response also. The action spectrum of the negative off-response resembles that of the negative on-response (P III), whereas the action spectrum of the positive off-response shows no participation of the 450nm-mechanism at all. We interpret this on-off asymmetry to mean that the positive off-response is generated by a quick return of the 555nm cone receptor potential, not present in the 450nm cones.

We conclude that there are at least two cone types in cat retina: One more numerous, more long wavelength sensitive and more rapid in its response; the other less numerous, more short wavelength sensitive and slower in its time course resembling in some respect rods. At a point beyond the receptor level (P III) but before (or at) the site of b-wave generation, opponency between these two cone mechanisms seems to occur.

Cyclic nucleotides, calcium, and the cat electroretinogram

We have examined whether putative changes in the concentration of cyclic guanosine monophosphate (cGMP) affect the rod a-wave response, recorded d.c. from arterially perfused cat eye-cups. Isobutylmethylxanthine (IBMX), an inhibitor of cGMP phosphodiesterase, delays the implicit time, prolongs the duration and increases the amplitude but not the sensitivity of the isolated

a-wave response. The effect is reversible and repeatable over two days. Cyclic GMP (dibutyril) was found to produce a somewhat similar but extremely weak effect, which develops slowly and only at relatively high concentrations (5 mM), although this is perhaps due to poor penetration. Low Ca^{++} solutions, EGTA buffered, mimic the effects of IBMX, although they seem to produce less changes in response time-course for comparable increases in amplitude. Prolonged exposure (e.g. four hour) to low Ca^{++} solutions were found to lead to depression of the response and, occasionally, has been accompanied by spontaneous electroretinographic oscillations. These effects have not yet been observed with prolonged IBMX exposures. The results suggest that cGMP may participate in the generation of electrical signals in the retina of the cat, having effects similar to but perhaps not identical with those of low Ca^{++} perfusates.

Significance to Biomedical Research and the Program of the Institute: In diagnosing and treating the diseases of the eye it may prove useful to know, on a detailed cell-by-cell basis, how the retina works, especially since many disease states have their origins at the cellular level, and treatments have as their targets particular classes of cells. A knowledge of normal retinal function provides a necessary substrate for interpreting and treating retinal dysfunction.

Proposed Course: This project will be continued.

NEI Research Program: Retinal and Choroidal Diseases - Visual Cells and Pigment Epithelium/Retinal Organization and Visual Adaptation

Experimental Subject or Tissue Source: Cat/Rabbit/Monkey

Research Objective: Etiology

Publications:

Famiglietti, E.V. Jr., and Kolb, H.: Structural basis for ON- and OFF-center responses in retinal ganglion cells. Science 194: 193-195, 1976.

Gouras, P.: Symposium on retinal circuitry (Preface). Invest. Ophthalmol. 15: 877-880, 1976.

Nelson, R., Kolb, H., Famiglietti, E.V., and Gouras, P.: Neural responses in the rod and cone systems of the cat retina: Intracellular records and Procion stains. Invest. Ophthalmol. 15: 946-153, 1976.

Nelson, R.: Cat cones have rod input: A comparison of the response properties of cones and horizontal cell bodies in the retina of the cat. J. Comp. Neurol. 172: 109-136, 1977.

Kolb, H., Famiglietti, E.V., and Nelson, R.: Neural connections in the inner plexiform layer of the cat's retina. In Yamada, E., and Mishima, S. (eds.): Structure of the Eye, Japan J. Ophthalmol., 1976.

Nelson, R., Famiglietti, E.V. Jr., and Kolb, H.: ON and OFF center ganglion cells in the retina of the cat: Intracellular staining. J. Neurophysiol. (in press).

Zrenner, E., and Gouras, P.: Spectral opponency and asymmetry between cone mechanisms in the cat electroretinogram (ERG). Society for Neuroscience Abstracts, 1977.

de Monasterio, F.M., and Gouras, P.: Spectral interactions in cells of perfused rabbit retina. ARVO Abstracts p. 62, suppl. to Invest. Ophthalmol., 16, No. 4, 1977.

Gouras, P., and de Monasterio, F.M.: Isobutylmethylxanthine, cyclic guanosine monophosphate, calcium and the electroretinogram of the perfused cat eye. ARVO Abstracts, p. 9, suppl. to Invest. Ophthalmol., 16, No. 4, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00148-04 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Cyclic Nucleotides and Vision

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Gerald J. Chader	Ph.D.	Head, Section on Retinal and Corneal Metabolism	LVR NEI
Other:	R. Theodore Fletcher	M.S.	Chemist	LVR NEI

COOPERATING UNITS (if any)
1) Gopal Krishna, Ph.D.; Laboratory of Chem. Pharmacology, NHLBI
2) Gustavo Aquirre, D.V.M., Dept. of Ophthalmology, Univ. Penn. Vet School Phila., Pa.

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.8	0.8	1.0

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

The enzymes of cyclic nucleotide metabolism are different in retina from those in other tissues and are active early in embryogenesis. High cyclic GMP levels are present in retinas of dogs with retinal degeneration indicating the possible involvement of cyclic GMP in the etiology of the degenerative process.

Project Description:

Objectives: To study the role of cyclic nucleotides in normal vision and their possible role in retinal disease.

Methods Employed: Photoreceptors of the retina are isolated by sucrose density gradient centrifugation and the activities of guanylate cyclase, phosphodiesterase and protein kinase are determined by standard biochemical methods. Cyclic nucleotide concentrations are measured by immunochemical titration after initial purification by column chromatography.

Major Findings: Cyclic nucleotides (e.g. cyclic GMP) and nucleoside-triphosphates (e.g. GTP) appear to be involved in the visual process. We have continued our investigation of 1) the phosphorylation of opsin by GTP 2) the formation of cyclic GMP from GTP through guanylate cyclase activity 3) the normal patterns of embryonic development of cyclic nucleotides and their enzymes of metabolism. In brief, we have found that the rod outer segment kinase enzyme which facilitates the phosphorylation of opsin by GTP exhibits specific requirements of cation concentration, pH, etc. for maximal expression of enzyme activity which are different from those in other tissues. Guanylate cyclase also appears to be different in rod outer segments than in other tissues and exhibits unique enzymatic characteristics. Cyclic GMP and cyclic AMP have different developmental patterns in retina and pigment epithelium which probably indicate different roles for the nucleotides in the tissues during embryogenesis.

In collaboration with Dr. G. Aquirre we have begun a study of the possible involvement of cyclic nucleotides in retinal degeneration in the Irish Setter. We have found extremely high cyclic GMP concentrations in the retinas of affected mature dogs while levels in control dogs (i.e. genetic carriers of the disease) are considerably lower. This may indicate a derangement in cyclic nucleotide metabolism in the disease.

Significance to Biomedical Research and the Program of the Institute: Study of the enzymes of cyclic nucleotide synthesis and degradation gives a better understanding of how the normal retina functions and could uncover the cause of at least one form of retinal degeneration.

Proposed Course: We will continue to study the possible role of cyclic GMP in both normal and abnormal retinal tissues with particular emphasis on metabolic changes in embryonic and early postnatal development.

NEI Research Program: Retinal and Choroidal Diseases - Developmental and Hereditary Disorders.

Experimental Subject or Tissue Source: Frog/Cow/Dog/Chick

Research Objective: Etiology

Publications:

Krishna, G., Krishnan, N., Fletcher, R., and Chader, G.: Effects of light on cyclic GMP metabolism in retinal photoreceptors. J. Neurochem. 27: 717-722, 1976.

Chader, G., Fletcher, R., O'Brien, P., and Krishna, G.: Differential phosphorylation by GTP and ATP in isolated rod outer segments of the retina. Biochemistry 15: 1615-1620, 1976.

Fletcher, R.: Cyclic nucleotides in the developing chick retina: Master of Science Thesis, American University, December, 1976.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00134-04 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Development and Function of Retina, Pigment Epithelium, and Cornea

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	Gerald J. Chader	Ph.D.	Head, Section on Retinal and Corneal Metabolism	LVR NEI
-	Barbara Wiggert	Ph.D.	Staff Fellow	LVR NEI
	Eileen Masterson	Ph.D.	Postdoctoral Fellow	LVR NEI
Other:	R.T. Fletcher	M.S.	Chemist	LVR NEI
	Julia Derr	B.S.	Biologist	LVR NEI

COOPERATING UNITS (if any)
Donald R. Bergsma, M.D., Staff Ophthalmologist, NEI:CB, Ralph Helmsen, Ph.D., Research Chemist, NEI:LVR; Marc Lewis, Ph.D., Research Chemist, NEI:LVR; Paul J. O'Brien, Ph.D., Research Chemist, NEI:LVR; Arnold Goldman, Ph.D. Staff Fellow NEI:LVR

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Laboratory of Vision Research

SECTION
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
3.8	3.5	0.3

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

1) Vitamin A receptors appear to be important in normal retina, pigment epithelium and cornea 2) A tissue culture system for studying outer segment phagocytosis has been established 3) The importance of the pentose shunt in early corneal development has been established.

Project Description:

Objectives: To study factors which affect the normal development and functioning of the retina-pigment epithelium unit and of the cornea.

Methods Employed: Vitamin A receptors in tissue extracts were determined by sucrose density gradient ultracentrifugation, gel filtration, spectroscopy and electrophoresis. Tissue culture techniques of cell dissociation, cloning and incubation were used in the phagocytosis experiments. Corneal studies were performed using standard methods for measuring glucose metabolism.

Major Findings: 1) Specific soluble receptors for vitamin A have been found in retina, pigment epithelium, and cornea. Two different receptors for retinol are present in retinas of most species, one of these is not seen in the retina of a patient with retinitis pigmentosa and during early fetal development in the cow indicating that this receptor species is predominantly associated with photoreceptor outer segments in vivo. These receptors may be involved in the normal development and/or vitamin A transport in ocular tissues.

2) In tissue culture, pigment epithelial cells from the chick embryo can phagocytize isolated bovine rod outer segments. We hope to use this as a model system for studying phagocytosis of outer segments under culture conditions. Several parameters affecting outer segments and pigment epithelial cells are currently under investigation in hopes of pinpointing the signals or factors controlling phagocytosis in vivo.

3) Factors affecting the onset of corneal transparency are under investigation in the chick embryo cornea. It has been found that pentose shunt activity appears concurrently with the process of detergescence. Highest shunt values are found after complete transparency is achieved. Diamide preferentially increases oxidation of glucose in the C-1 position indicating an elevation of pentose shunt activity with this agent. Thus, the enzymes for pentose shunt activity are present early in corneal development and are probably necessary in maintaining reduced glutathione needed for corneal transparency.

Significance to Biomedical Research and the Program of the Institute: Retinal dystrophy and keratomalacia are diseases of the retina and cornea respectively in which vitamin A appears to be involved. It is hoped that the study of factors which affect the development of the tissues will indicate areas of biochemical research which will ultimately lead to prevention of the diseases.

Proposed Course: Factors both external (e.g. hormones, vitamins) or internal (e.g. tissue receptors) which affect the development and function of ocular tissues will continue to be investigated.

NEI Research Program: Retinal and Choroidal Diseases - Developmental and Hereditary Disorders

Project No. Z01 EY 00134-04 LVR

Experimental Subject or Tissue Source: Cow/Rat/Monkey/Pig/Chicken/Human

Research Objective: Etiology

Publications:

Redfern, N., Israel, P., Bergsma, D., Robison, W., Whikehart, D., and Chader, G.: Neural retinal and pigment epithelial cells in culture: Patterns of differentiation and effects of prostaglandins and cyclic AMP on pigmentation. Exp. Eye Res. 22: 559-568, 1976.

Wiggert, B., Bergsma, D., and Chader, G.: Retinol receptors of the retina and pigment epithelium. Exp. Eye Res. 22: 411-418, 1976.

Bergsma, D., Wiggert, B., Funahashi, M., Kuwabara, T., and Chader, G.: Vitamin A receptors in normal and dystrophic human retina. Nature 265: 66-67, 1977.

Helmsen, R., Wiggert, B., and Chader, G.: A possible receptor for retinol in corneal epithelium. Exp. Eye Res. 24: 213-214, 1977.

Wiggert, B., Bergsma, D., Helmsen, R., Alligood, J., Lewis, M., and Chader, G.: Retinol receptors in corneal epithelium, stroma and endothelium. Biochim. Biophys. Acta 491: 104-113, 1977.

Abe, T., Wiggert, B., Bergsma, D., and Chader, G.: Vitamin A receptors: Comparison of retinol binding to serum retinol-binding protein and to tissue receptors in chick retina and pigment epithelium. Biochim. Biophys. Acta (in press).

Wiggert, B., Bergsma, D., Lewis, M., Abe, T., and Chader, G.: Vitamin A receptors: Characteristics of retinol binding in chick retina and pigment epithelium. Biochim. Biophys. Acta (in press).

Wiggert, B., Bergsma, D., Lewis, M., and Chader, G.: Vitamin A receptors: Retinol binding in neural retina and pigment epithelium. J. Neurochemistry (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00016-10 LVR

PERIOD COVERED

July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Protein Synthesis in the Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Paul J. O'Brien Ph.D. Research Chemist LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH

Laboratory of Vision Research

SECTION

Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION

National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.0	0.3	0.7

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

A small portion of the rhodopsin in bovine rod outer segments can be labeled with galactose and fucose in vitro. These sugars are probably added in the outer segment and may play a role in phagocytosis. Various precursors of rhodopsin can be incorporated by both bovine and canine retinas thereby permitting examination of possible defects in dystrophic animals.

PHS-6040

Project Description:

Objectives: The renewal of photoreceptor cell outer segments is a continuous process which is impaired in some pathological conditions such as progressive degeneration or developmental anomalies of the retina. The purpose of this project is the elucidation of the biochemical events involved in renewal, especially the synthesis of protein in the retinas of both cow and dog.

Methods Employed: Ordinary biochemical techniques were used, such as incubation of retinas, cell fractionation, isolation of rod outer segments by density gradient centrifugation, detergent extraction and purification of rhodopsin by column chromatography and gel electrophoresis.

Major Findings: Protein synthesis with dog retinas in vitro gave gel electrophoresis labeling patterns similar to those seen with bovine retina, thereby establishing the utility of the methods for future work with dog retinas. Puromycin, an inhibitor of protein synthesis, produced different inhibition patterns in bovine retina depending on the radioactive precursor used. The degree of inhibition reflected the sequence of addition of sugar residues to rhodopsin. Both fucose and galactose were unaffected by puromycin and appear to be added in the outer segments, probably to a small portion of the rhodopsin.

Significance to Biomedical Research and the Program of the Institute: The ability to measure protein synthesis in normal dog retinas will permit similar measurements in dogs with inherited retinal degenerations in an attempt to detect specific lesions. The presence of galactose and fucose on a small fraction of the outer segment rhodopsin may provide a marker to facilitate phagocytosis of shed disc membranes. Defects in the addition of these sugars could account for degenerative processes. Animal models, such as dogs, provide the means of testing this hypothesis.

Proposed Course: The incorporation of various precursors of rhodopsin, especially galactose and fucose, will be studied in several animal models of retinal degeneration to determine whether specific abnormalities can be identified.

NEI Research Program: Retinal and Choroidal Diseases - Developmental and Hereditary Disorders/Visual Cells and Pigment Epithelium

Experimental Subject or Tissue Source: Bovine/Canine

Research Objective: Etiology

Publications:

O'Brien, P.J.: Differential effects of puromycin on the incorporation of precursors of rhodopsin in bovine retina. Biochemistry 16: 953-958, 1977.

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00015-12 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Synthesis of Sugar-Containing Polymers in Retina

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI: Paul J. O'Brien Ph.D. Research Chemist LVR NEI

COOPERATING UNITS (if any)

None

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Md. 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
0.8	0.5	0.3

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☐ (a) HUMAN SUBJECTS ☐ (b) HUMAN TISSUES ☒ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

Both galactose and fucose are incorporated into rhodopsin by isolated rod outer segment preparations. These sugars might provide specific markers to permit the pinching off of new disc membranes or the shedding of old discs with resultant phagocytosis by the pigment epithelium during the course of photoreceptor outer segment renewal. Retinyl phosphate, possibly provided by the pigment epithelium, may act as a carrier of galactose.

Project Description:

Objectives: Many interactions between macromolecules and cell membranes are mediated by the sugar molecules bound to one of the interacting surfaces. In the process of renewal of photoreceptor outer segment disc membranes, rhodopsin, a glycoprotein, must be transported from the inner segment and incorporated into disc membranes with a specific orientation in space. This project was designed to determine where and when sugars are added to the polypeptide and what role they play in the transport and assembly of rhodopsin into disc membranes and in the process of shedding and phagocytosis of disc membranes.

Methods Employed: Ordinary biochemical techniques were used, such as cell fractionation, isolation of rod outer segments by density gradient centrifugation, detergent extraction and purification of rhodopsin by column chromatography, and incubation of outer segment preparations.

Major Findings: Isolated photoreceptor outer segment preparations catalyze the transfer of galactose and fucose from the appropriate sugar nucleotides to rhodopsin. The transfer of galactose, but not of fucose, appears to be mediated by a lipid carrier, possibly retinyl phosphate. The evidence suggests that the pigment epithelium supplies an essential factor for the transfer reactions.

Significance to Biomedical Research and the Program of the Institute: The addition of galactose and fucose to rhodopsin might provide a marker to permit the pigment epithelium to distinguish shed photoreceptor tips from intact outer segments. This process appears to be under the control of the pigment epithelium, perhaps by providing retinyl phosphate. Certain animal models of retinal degeneration manifest a defect in the pigment epithelium. This mechanism could be defective either in animal models or in human retinal degenerations.

Proposed Course: Attempts will be made to demonstrate the synthesis of retinyl phosphate and retinyl phosphate galactose in the pigment epithelium or photoreceptors. If successful, retinyl phosphate galactose will be used as a donor of galactose residues to rhodopsin. These pathways will be assayed in animals with retinal degenerations.

NEI Research Program: Retinal and Choroidal Diseases - Developmental and Hereditary Disorders/Visual Cells and Pigment Epithelium

Experimental Subject or Tissue Source: Bovine

Research Objective: Etiology

Publications:

O'Brien, P.J.: Incorporation of mannose into rhodopsin in isolated bovine retina. Exp. Eye Res. 24: 449-458, 1977.

Bok, D., Hall, M.O., and O'Brien, P.: The biosynthesis of rhodopsin as studied by membrane renewal in rod outer segments. In Brinkley, B.R. and Porter, K.R. (eds.): International Cell Biology, 1976-1977. New York, Rockefeller University Press (in press).

SMITHSONIAN SCIENCE INFORMATION EXCHANGE PROJECT NUMBER (Do NOT use this space)	U.S. DEPARTMENT OF HEALTH, EDUCATION, AND WELFARE PUBLIC HEALTH SERVICE NOTICE OF INTRAMURAL RESEARCH PROJECT	PROJECT NUMBER Z01 EY 00024-03 LVR

PERIOD COVERED
July 1, 1976 to September 30, 1977

TITLE OF PROJECT (80 characters or less)

Intermediary Metabolism of the Cornea

NAMES, LABORATORY AND INSTITUTE AFFILIATIONS, AND TITLES OF PRINCIPAL INVESTIGATORS AND ALL OTHER PROFESSIONAL PERSONNEL ENGAGED ON THE PROJECT

PI:	David R. Whikehart Ph.D.	Senior Staff Fellow	LVR NEI
Other:	Henry F. Edelhauser Ph.D.	Professor, Medical College of Wisconsin, Milwaukee	
	R. Theodore Fletcher M.S.	Chemist	LVR NEI

COOPERATING UNITS (if any)

Dept. of Physiology, Medical College of Wisconsin, Milwaukee

LAB/BRANCH
Laboratory of Vision Research

SECTION
Section on Retinal and Corneal Metabolism

INSTITUTE AND LOCATION
National Eye Institute, NIH, Bethesda, Maryland 20014

TOTAL MANYEARS:	PROFESSIONAL:	OTHER:
1.5	1.3	0.2

CHECK APPROPRIATE BOX(ES)

☐ (a) HUMAN SUBJECTS ☒ (b) HUMAN TISSUES ☐ (c) NEITHER

☐ (a1) MINORS ☐ (a2) INTERVIEWS

SUMMARY OF WORK (200 words or less - underline keywords)

This investigation concerns itself with the biochemical mechanisms that control corneal hydration (deturgescence). The influence of bicarbonate, glucose, glutathione and adenosine on intracellular levels of reduced and oxidized glutathione in the corneal endothelium along with swelling rates have been investigated via perfusion. All of these compounds have been shown to be effective, in varying degrees, in preventing swelling. In combination they appear to actually promote deswelling. Endothelial cells grown in culture are being assayed for levels of glutathione and cyclic nucleotides. Levels of glutathione peroxidase have been assayed and show higher activity levels in the corneal endothelium than in the corneal epithelium.

Project Description:

Objectives: This project has been initiated to elucidate the biochemistry of corneal deturgescence and to suggest mechanisms for its control. Its immediate objectives are: 1) to determine the location, accessibility and reactivity of specific areas of the plasma membrane of the corneal endothelium to cytoplasmic, sulfhydryl/disulfide metabolites, 2) to measure intracellular levels of adenosine, 3) to establish better conditions for tissue culture and/or eye bank storage by observing what metabolites or activators produce optimal ATPase activity

Methods Employed: Major sources of tissue have been rabbit and bovine eyes. Human tissue has also been studied on a limited basis. Extremely sensitive and accurate techniques of assay employing double-beam spectrophotometry and gas liquid chromatography have been employed. These procedures assay intermediates at the nanogram level. Tissue cultures of endothelial cells have been initiated from micro-dissected endothelial/Descemet's layer buttons from rabbits. Perfusions of rabbit corneas have been accomplished with the aid of the specular microscope to measure corneal swelling rates. Glutathione peroxidase was assyed by an indirect kinetic method employing glutathione reductase. Cyclic nucleotides have been assayed by radioimmunoassay.

Major Findings:

Perfusion of rabbit corneas

Certain metabolites have been implicated in the activation of the sodium pump: glutathione, adenosine and bicarbonate. However, the exact effect of these substances on ATPase has not been established in the corneal endothelium. Investigation of endogenous levels of reduced and oxidized glutathione in endothelial tissue of rabbit cornea showed remarkable differences when perfused by different compounds. Endothelial cells had a lower amount of total glutathione with bicarbonate or lactate Ringer's media compared to fresh, unperfused controls. Adenosine, reduced and oxidized glutathione caused normal to elevated levels of total glutathione, under which conditions the cornea remained stable (i.e. did not swell) for up to 5 hrs. High levels of oxidized glutathione (30-70% of the total) were found in all perfusion experiments and in the control. The discovery of this high level of oxidized glutathione implies that the hexose monophosphate shunt (highly active in corneal epithelial tissue) may not be very active in corneal endothelium. It points to the existence of a pool of oxidized glutathione in that tissue. The results of this study have been submitted to Investigative Ophthalmology and Visual Science for publication.

Glutathione peroxidase activity

Concomitantly, it has been demonstrated that the activity levels of glutathione reductase in bovine and rabbit corneal endothelia are 8-20 times lower than the corresponding levels in corneal epithelia. I have shown that the converse is true for glutathione peroxidase activity in bovine

corneal tissues (i.e. endothelium > epithelium). The implication of these studies is that the pentose shunt is comparatively low in activity in the endothelium allowing relatively high amounts of oxidized glutathione to exist in the cells. Since I have already been able to show that the ratio of oxidized to reduced glutathione in fresh bovine, rabbit and human corneal endothelial tissues is high compared to most other tissues including corneal epithelium, this completes the picture of a redox state of glutathione in the corneal endothelium which highly favors oxidation. A manuscript is in preparation.

Cyclic nucleotide levels

This work is still in progress. Both the levels in fresh tissue (corneal epithelium and endothelium) and in tissue cultures of the corneal endothelial cells of the rabbit are being examined for cyclic GMP and cyclic AMP. To date, substantial levels of cyclic GMP have been found in both fresh tissues and in tissue cultures.

Significance to Biomedical Research and the Program of the Institute: Corneal disease involving disturbances in the proper hydration (deturgescence) of the cornea (resulting in cloudy, impaired vision) are thought to be the result of metabolic dysfunction (degeneration). Such dysfunctions are the result of damage from transplants, storage conditions (cornea banks), inflammatory reactions and inherent dystrophies. Presently, however, the normal metabolic functions associated with the hydration pump(s) and its controls have not been adequately described. Since glutathione is known to strongly promote deturgescence, its endogenous level is quite important. The high level of oxidized glutathione, found in the corneal endothelium, implies the existence of a metabolic scheme quite modified from that of corneal epithelium and of a number of other cellular tissues. It has only recently been discovered that the oxidized form is equally as effective as the reduced form in stimulating the pump(s), but it is not understood how this takes place. The possibility that cyclic nucleotides may also play a role in controlling the activity of the deturgescent pump is of equal potential importance to the discovery of the role of glutathione.

Proposed Course: The study of the deturgescent mechanism in the corneal endothelium, in view of its investigative history, ought to be to elucidate ATPase activity and to describe the role of substances known or suspected to maintain ATPase at a functional level of activity. One question to address to this study is, therefore, "what is the role of sulfhydryl and/or disulfide groups in relation to ATPase activity in the corneal endothelium in promoting deturgescence?" Solving these questions and determining the role of SH/SS will require the location of SH groups on the endothelial cell plasma membrane. Membrane sulfhydryls will be located by the utilization of impermeant meleimides and N-ethyl maleimide.

Adenosine's influence must also be described. The determination of the endothelial cell content of adenosine is already in progress in my laboratory using adenosine deaminase for the assay. Its effects will be more directly studied when endothelial ($Na^+ + K^+$)-ATPase is prepared from the plasma membrane.

Tissue cultures of corneal endothelial cells will be studied to determine how the cell's levels of activity of $(Na^+ + K^+)$-ATPase are effected by various metabolites such as glutathione, adenosine and cyclic nucleotides. Assay work for this study is already in progress.

NEI Research Program: Corneal Diseases - Corneal Edema, Dystrophies, and Inherited Disorders

Experimental Subject or Tissue Source: Bovine/Rabbit/Human

Research Objectives: Etiology, Treatment

Publications:

Redfern, N., Israel, P., Bergsma, D., Robison, Jr., W.G., Whikehart, D., and Chader, G.: Neural retinal and pigment epithelial cells in culture: Patterns of differentiation and effects of prostaglandins and cyclic-AMP on pigmentation. Exp. Eye Res. 22: 559-568, 1976.

Whikehart, D.R., and Hess, H.H.: Properties of liposomes with a phospholipid ratio similar to that of retinal rod outer segment membranes: Interaction with opsin and other proteins. Exp. Eye Res. 24: 279-289, 1977.

OFFICE OF BIOMETRY AND EPIDEMIOLOGY

ANNUAL REPORT
NATIONAL EYE INSTITUTE
July 1, 1976 - September 30, 1977

REPORT OF THE CHIEF, OFFICE OF BIOMETRY AND EPIDEMIOLOGY

Fred Ederer

The Office of Biometry and Epidemiology was strengthened considerably, both scientifically and administratively, during the past year when Dr. Daniel Seigel, who has had a distinguished career as a biostatistician, joined the staff as Deputy Chief. Dr. Seigel, whose most recent position had been Director, Epidemiology and Biometry Research Program, National Institute of Child Health and Human Development, was also appointed as Head, Section on Clinical Trials and Natural History Studies, replacing Fred Ederer in that capacity. With Dr. Seigel's appointment, Mr. Ederer relinquished his position as Head, Section on Clinical Trials and Natural History Studies, and was able to increase attention to his role as Acting Head of the Epidemiology Section. The main functions of the Office are:

1. To develop and carry out studies of human populations directed toward causation, prevention, and treatment of eye disease and vision disorders, with emphasis on the major causes of blindness. This includes studies of incidence and prevalence in defined populations, prospective and retrospective (case-control) studies of risk factors, natural history studies, clinical trials, genetic studies, and studies to evaluate diagnostic procedures. The incidence and prevalence studies, apart from providing epidemiologic research leads, make information available for assessing the magnitude of the national eye disease problem.

2. To provide biometric and epidemiologic assistance to National Eye Institute intramural research scientists and, to the extent feasible, to vision researchers elsewhere.

3. To disseminate information about biometric and epidemiologic methods to those in vision research.

A large part of the Office's effort has been devoted to providing scientific support to clinical trials and other epidemiologic studies conducted under NEI research contracts, mainly the Diabetic Retinopathy Study, the Diabetic Retinopathy Vitrectomy Study, the Diabetic Retinopathy Study II, and the Framingham Eye Study. Major research papers were published on the Framingham Eye Study, and a final report was submitted on the Biostatistical Analysis of the Collaborative Glaucoma Study contract. In addition, professional staff of the Office developed further studies based on existing epidemiological data from the Model Reporting Area on Blindness and the Framingham Eye Study. From these latter efforts, a paper was published showing a positive association between the prevalence of senile cataract and residence in areas of high sunshine.

A specific function of the Office of the Chief is to disseminate information about biometric and epidemiological methods to those engaged in vision research. In recent years activities in this area have included the conduct of a workshop on the randomized clinical trial before the Association of University Professors of Ophthalmology and the publication of methodological papers in ophthalmic and optometric journals. During the past year, the Office organized and conducted a course on methods of clinical research at the 1976 meeting of the American Academy of Ophthalmology and Otolaryngology. Another such course is planned for the 1977 meeting.

For the past several years the Office has succeeded in recruiting clinical associates with an interest in clinicoepidemiologic research to participate in its programs. These clinical associates, who usually spend two years with the OBE, are either recent graduates of ophthalmology residency programs or physicians planning to enter such programs and, if the latter, they are provided during their tenure with some ophthalmology training. The clinical associates provide an essential ophthalmologic complement to the Office's biometric and epidemiologic expertise. Moreover, as these people advance in their academic careers, they are able not only to apply their biometric and epidemiologic knowledge to their own work, but also to disseminate it to their colleagues in vision research.

As a member of a subcommittee of the NIH Clinical Trials Committee, Mr. Ederer participated in the planning of the NIH Conference on Clinical Trials Methodology scheduled for October 1977.

Mr. Ederer represented the National Eye Institute in presenting papers at a national meeting of the American Association of Workers for the Blind and the Conference on Impaired Vision in Childhood in Jerusalem. He also presented papers at meetings of the American Statistical Association and the International Epidemiological Association.

Epidemiology Section

Office of Biometry and Epidemiology

Mr. Ederer continued to serve as Acting Head of this Section. The functions of the Section are to develop and conduct a program of epidemiological research in eye disease, with particular emphasis on diseases which are leading causes of blindness and visual impairment in the United States, and to provide consultation on epidemiological problems to others in vision research. The epidemiological studies emphasize investigations to uncover clues about etiology and pathogenesis, such as prevalence surveys, case control studies, population genetic studies, and studies directed at improving diagnostic methods.

A study of the relationship of sunlight and cataract was published. Cataract prevalence data from two large U.S. sources were divided according to small geographic areas for which average annual sunlight hours were determined from a map prepared by the U.S. Weather Bureau. Several noncataract disease controls were chosen from the same geographic locations. It was found that the cataract-to-control ratios for persons aged 65 years or older were significantly larger in locations with large amounts of sunlight compared to those in locations with small amounts ($p < .05$). Discussion of some possible biases in the data led to the conclusion that the biases, if they exist, are probably not large. The report suggests, however, that more research should be done before the association between sunshine and cataract is considered established.

Mrs. Hiller has continued to collaborate with Dr. Matthew D. Davis, University of Wisconsin, in a study of mortality in diabetics with varying degrees of severity of retinopathy. Survival patterns are also being studied by age at diagnosis of diabetes, visual acuity, and duration of diabetes. A first draft of the material, methods, and results sections of a manuscript has been prepared.

A contract to analyze data collected in the Collaborative Glaucoma Study was completed. Major risk factors for glaucoma visual field defects (GVFD) were evaluated. Though subgroups could be identified whose risk of GVFD were increased by as much as ten-fold, they represent only a small fraction of the total population. The conclusion is that these risk factors do not afford high predictability for such disorders. The contractor was encouraged to publish those findings now available.

Analysis of the Framingham Eye Study continued. Two major initial reports were published. Grading of stereo fundus photographs for disc and macula abnormalities (1,500 patients) and diabetic retinopathy (500 patients) will be completed this year. A statistical monograph is being prepared under subcontract by the Biostatistics Center, George Washington University.

An Advisory Group on Epidemiology comprised of personnel from the Clinical Branch, Laboratory of Vision Research, and Office of Biometry and Epidemiology was formed to advise the Acting Head, Epidemiology Section, on research activities in epidemiology. The group met twice to review the Section's activities

and to advise the Acting Section Head on various research proposals Several studies that were assigned a high priority by the Advisory Group are now being carried out by various staff members of the Office of Biometry and Epidemiology.

The Health and Nutrition Examination Survey (HANES), of the National Center for Health Statistics (NCHS), in which staff of the Office of Biometry and Epidemiology participated in 1971-1972, has issued several monographs giving information about the plan and operation of the survey and about monocular visual acuity findings. Further HANES publications are being planned.

A number of visits were made by Mr. Ederer and his staff to the National Center for Health Statistics to discuss plans for analyzing the data from the Detailed Ophthalmologic Examination portion of the Health and Nutrition Examination Survey. The NCHS has assured the OBE that a copy of the computer tape including the eye data will soon be made available to the NEI for epidemiologic analyses.

Section on Clinical Trials and Natural History Studies

Office of Biometry and Epidemiology

The principal activities of this Section are the conduct of randomized clinical trials on the treatment and prevention of eye disorders, the conduct of nonrandomized studies on their natural history, and the provision of consultation to colleagues in other parts of the Institute who are also active in clinical trials research. Of these, the scientific management of clinical trials, conducted by National Eye Institute project teams, was the dominant activity during FY 1977. Project teams are led by a staff member of the Office of Biometry and Epidemiology and include expertise in biostatistics, epidemiology, ophthalmology, computer science, contract management, and public information.

The Diabetic Retinopathy Study (DRS), following publication of its first report in 1976, has continued follow-up of its patients under a modified protocol allowing, but not requiring, treatment of eyes originally assigned to no treatment if they meet certain high-risk criteria. Large numbers of patients have been followed for three years, and the first fifth-year follow-up visit has occurred. The DRS has concentrated its analyses on determining the effect of photocoagulation on the development and progression of retinopathy, and is now attempting to determine the role of photocoagulation in selected subgroups of the patient population.

A monograph giving details of study design, methods, and baseline characteristics of patients is being prepared for publication, as is a methodologic paper on fundus photograph grading. The Study has entered an analytic phase where emphasis will be placed on developing detailed descriptions of the natural history of diabetic retinopathy and elaborating on the effects of photocoagulation treatment. The DRS Project Team has been actively involved in these activities.

The Diabetic Retinopathy Study Phase II (DRS II) is a multicenter randomized clinical trial designed to answer some of the major questions not addressed by the DRS and other studies. The important issues are optimum stage of disease to initiate photocoagulation, the effectiveness of photocoagulation on diabetic maculopathy, and whether aspirin administered at various stages of diabetic retinopathy can prevent or retard the progression of this disease. Funding of the study should begin in the fall of 1977, with initial year's goal to be completion of the study design and preparation of a detailed manual of operations. Recruitment of patients should begin by September of 1978.

The Diabetic Retinopathy Vitrectomy Study (DRVS) is a clinical trial involving treatment of a more advanced stage of diabetic retinopathy in which blindness due to hemorrhage into the vitreous has occurred. The surgical procedure being examined is vitrectomy using an instrument combining cutting, suction, and infusion of a replacement solution. Eligible eyes are assigned to either vitrectomy within the first six months of a vitreous hemorrhage or to a "late" vitrectomy group in which vitrectomy is performed at one year following hemorrhage in those eyes still suitable. In October 1976 recruitment of patients was begun for those clinical centers meeting requirements for certification of clinic personnel.

With improvements in the design of lenses for intraocular implantation, the number of such implants has been increasing at an exponential rate. Many feel that it is important at this time to evaluate carefully the efficacy of such implants in patients undergoing cataract extraction and to assess the risks as well.

As a first step toward evaluating intraocular lens implants, the National Eye Institute will attempt to summarize the experience that ophthalmologists have had in recent clinical practice. The records of several surgeons will be analyzed to see whether they can provide answers to research questions that are of interest. For findings from study of such records to be credible, it will be necessary to demonstrate that data from all patients treated during a defined interval are included, that follow-up was maintained on a large proportion of cases, and that significant medical data have been carefully recorded.

Demonstrating that these elements are present constitutes the work scope of a contract which was awarded in the fall of 1977. If in the first year of the contract the feasibility of a high quality retrospective study is established, the contract will be extended to analyze and report on the benefits and risks of intraocular lens implants. Consideration is also being given to more rigorous research designs, such as prospective controlled studies, perhaps including randomization, but it is not yet clear that these are feasible.

A standardized visual acuity box to assure uniformity of visual acuity measurement was developed by Dr. Ferris. Arrangements were made for its manufacture, and it has been distributed to clinics participating in ongoing clinical trials.

The Section has accepted responsibility for reviewing clinical trials protocols developed under NEI research grants. This has turned out to be a useful administrative mechanism by which the Institute helps to assure the quality of grant-sponsored clinical trials and offers the additional benefit of promoting communication on activities of shared interest between staff within the Section and staff from extramural programs.

The Section has also been involved in cooperative arrangements involving other governmental components. Dr. Lawrence Rand has served on an advisory group to NIAMDD to determine the feasibility of developing a clinical trial on the effect of metabolic control on the development of the vascular complication of diabetes mellitus. Dr. Seigel chairs the Advisory Committee on Biostatistics and Epidemiology to the Bureau of Drugs for the FDA. He also represents the NEI at the NIH Committee on Clinical Trials.

Biometry Section

Office of Biometry and Epidemiology

The Biometry Section, previously called the Section on Mathematical Statistics and Computer Applications, has chosen its new name to reflect more accurately the emphasis given to support and development of biostatistical programs and analysis. Activities during this year continue to include consulting and collaborating, both within and outside of NEI, in applied statistics and epidemiologic investigations.

Dr. Roy Milton, together with Drs. Robert Frank, Barry Coller, and Harvey Gralnick, received the Fight for Sight Citation "For Achievement in Clinical Vision Research" at the 1977 annual meeting of the Association for Research in Vision and Ophthalmology (ARVO), for collaborative effort in their 1976 ARVO paper, "Von Willebrand Factor and Effect on Platelet Aggregation of Plasma from Diabetics with Retinopathy." This and subsequent work will appear in the Annals of Internal Medicine.

Dr. Milton, with Dr. James Ganley, formerly Senior Staff Fellow in OBE and now at the University of Arizona, Department of Ophthalmology, presented a paper at the 1977 ARVO meeting, "Risk of glaucoma in myopia: a population study." He also participated in a round table discussion on distribution of statistical software at the Computer Science and Statistics Tenth Annual Symposium on the Interface.

Dr. Milton continued to consult with Dr. Douglas Gaasterland, NEI Clinical Branch, on studies of the parameters of aqueous humor dynamics, and is coauthor of three papers submitted for publication.

The Biometry Section is assisting Dr. Arin Chatterjee, Christian Medical College, Ludhiana, India, in a cataract etiology study. Data collection is completed and analysis has begun. The Section continues to monitor the Framingham Eye Study contract through activities of the project officer, coding and protocol maintenance, and exploratory data analysis.

Dr. Karen Yuen completed two years with the Section under the Intergovernmental Personnel Act, during which time she engaged in intramural consulting and research in statistical methodology. Dr. Yuen is now in the Department of Mathematics, University of Windsor.

The Section's resources in statistical computing were enhanced by acquisition of a Tektronix 4051 graphics terminal with stand-alone computing abilities and by hiring a programmer. These resources will improve the Section's ability to provide support for increased activity in epidemiologic analysis, especially of Framingham Eye Study data.

CONTRACT NARRATIVE

BOSTON UNIVERSITY (NIH-NEI-72-2112)

Title: Framingham Eye Study

Principal Investigator: Howard Leibowitz, M.D.

Current Fund Allocation: $672,826 for the period July 1, 1972 through December 31, 1977.

Objectives: The aim of this epidemiologic investigation is to identify individuals among the Framingham Heart Study cohort who at the present time have a disease or condition related to one or more of the four most common causes of adult blindness, i.e., senile cataract, senile macular degeneration, chronic simple glaucoma, and diabetic retinopathy. In addition to determining the prevalence of these diseases, past measurements from the Framingham Heart Study will be related to present disease status in an effort to identify risk factors.

Major Findings: An ocular examination according to a standard protocol was made on the survivors of the original Framingham Heart Study cohort. Patient examinations were completed in February 1975 with 2,675 individuals examined. This includes 84% of the cohort still residing in the Framingham area. The first two major reports[3,4] included findings of significant association between senile cataract and increases in serum phospholipids, casual blood sugar, blood pressure, and age. Senile macular degeneration was found to be associated with increased blood pressure, ventricular hypertrophy, history of lung infection, aging-related factors, and sex. Prevalence in this population was 3% for open angle glaucoma and diabetic retinopathy, 9% for senile macular degeneration, and 15% for cataract. Other findings are reported in the publications. Grading of stereo fundus photographs for disc and macular abnormalities (1,500 patients) and diabetic retinopathy (500 patients) will be completed this year. A statistical monograph is being prepared under subcontract to the Biostatistics Center, George Washington University.

Significance to Biomedical Research and the Program of the Institute: The four eye diseases under consideration are the leading causes of adult blindness in this country today. It will be very helpful to identify factors possibly associated with increased risk of these diseases, as a guide to prevention. The Study has been designed with this objective in mind. Prevalence data for this age group (52-85) in this community will be a useful by-product.

Proposed Course: Several additional major reports, including the statistical monograph, will be completed early in FY 78. A final data tape including summary of fundus photograph grading will be prepared to conclude data collection and editing. Contract completion is expected in FY 78.

NEI Research Program: Retinal and Choroidal Diseases - Macular Diseases/Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities; Cataract - Senile Cataract; Glaucoma..

Experimental Subject or Tissue Source: Human

Research Objective: Etiology

CONTRACT NARRATIVE

Title: Diabetic Retinopathy Study (DRS)

Current Fund Allocation:

Objectives: The Diabetic Retinopathy Study (DRS) is a multicenter clinical trial to evaluate the efficacy of photocoagulation (argon laser and xenon arc) in the treatment of proliferative diabetic retinopathy. This randomized, controlled study involves over 1,700 patients enrolled in the trial at fifteen medical centers.

Major Findings: Following publication of its first report showing photocoagulation to be effective in reducing the rate of severe visual loss in patients with proliferative diabetic retinopathy and identifying certain high-risk retinopathy characteristics, the DRS has continued follow-up of its 1,700 patients under a modified protocol allowing, but not requiring, treatment of eyes originally assigned to no treatment if they met these high-risk criteria. Large numbers of patients have been followed for three years, and the first 5th-year follow-up visit has occurred. The DRS has concentrated its analyses on determining the effect of photocoagulation on the development and progression of retinopathy, and is now attempting to determine the role of photocoagulation in eyes with milder stages of retinopathy than those described in its first publication.

A baseline monograph giving details of study design and procedures, and presenting baseline data on patient characteristics is being prepared for publication, as is a methodologic paper on fundus photographic grading.

The DRS has entered an analytic phase where emphasis will be placed on developing detailed descriptions of the natural history of diabetic retinopathy and elaborating on the effects of photocoagulation treatment.

The DRS realizes its obligation to disseminate its findings to the medical community and has staffed exhibits at the American Diabetes Association annual meetings and at the American Academy of Ophthalmology and Otolaryngology meetings. The Study plans to continue this and other types of educational effort and attempts are being made to cooperate with non-DRS investigators in developing guidelines for photocoagulation treatment of diabetic retinopathy in clinical practice.

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy, uncommon only a few decades ago, is now a leading cause of blindness and visual disability in the United States. There is a critical need to find and scientifically evaluate treatments which will reduce the risk of blindness or visual impairment from the ocular complications of diabetes. Although photocoagulation is widely used as a treatment, adequate evidence of its efficacy is now based on carefully documented research findings.

Proposed Course: Follow-up of all DRS patients continues and monitoring of accumulating data is performed at three-month intervals. This additional follow-up, as well as further data analysis, is required for a complete assessment of photocoagulation as used in the DRS, and the results of these analyses will be presented in future study publications.

NEI Research Program: Retinal and Choroidal Diseases - Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities

Experimental Subject or Tissue Source: Human

Research Objective: Treatment

Publications:

The Diabetic Retinopathy Study Research Group: Preliminary report on effects of photocoagulation therapy. Am. J. Ophthalmol. 81: 383-396, 1976

CONTRACT NARRATIVE

Title: Diabetic Retinopathy Vitrectomy Study (DRVS)

Current Fund Allocation: $287,000 for the period June 25, 1976 through June 26, 1977 (six new clinics).

Objectives: The DRVS is a multicenter clinical trial to: (a) evaluate vitrectomy performed in the first six months after vitreous hemorrhage secondary to diabetic retinopathy, as compared to the more usual practice of waiting twelve months after vitreous hemorrhage to remove the vitreous, and (b) to collect natural history data on patients who have diabetic retinopathy with extensive formation of abnormal blood vessels and/or early retinal detachment, but without extensive vitreous hemorrhage.

Major Findings: In June 1976, six additional Clinical Centers were added. In October 1976, recruitment of patients was begun by those Centers that had completed required certification procedures specified by the Manual of Operations. All but one Center were enrolling patients by May 1977.

Significance to Biomedical Research and the Program of the Institute: Diabetic retinopathy is one of four major causes of adult blindness and differs from the other three (macular degeneration, glaucoma, cataract) in that it affects a younger population. A major cause of this blindness is vitreous hemorrhage. Vitrectomy has been shown to be of some benefit to individuals who have had a severe vitreous hemorrhage for at least one year, and it is thought that diabetic blindness can be further reduced if vitrectomy is performed at an earlier date. This presents an ideal opportunity for the National Eye Institute to organize scientific talents to answer a significant medical question.

Proposed Course: The first six months of the period constitute a trial period in which the capability of all Clinical Centers to recruit sufficient patients is being examined. The Coordinating Center is processing the results, which will be reviewed by the Data Monitoring Committee.

The Reading Center is grading baseline fundus photographs and will be processing posttreatment photographs as they become available.

NEI Research Program: Retinal and Choroidal Diseases - Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities/Vitreous Humor

Experimental Subject or Tissue Source: Human

Research Objective: Etiology, Treatment

Publications:

None

PUBLICATIONS

Office of Biometry and Epidemiology

Allen, D.C.: Statistical and methodological considerations for vision screening. Am. J. Optom. Physiol. Opt. 53: 677-681, 1976.

Hiller, R., Giacometti, L., and Yuen, K.: Sunlight and cataract: An epidemiologic investigation. Am. J. Epidemiol. 105: 450-459, 1977

Kahn, H.A., Leibowitz, H.M., Ganley, J.P., Kini, M.M., Colton, T., Nickerson, R.S., and Dawber, T.R.: The Framingham Eye Study. 1. Outline and major prevalence findings. Am. J. Epidemiol. 106: 17-32, 1977.

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Milton, R.C., Ganley, J.P., and Lynk, R.H.: Variability in grading diabetic retinopathy from stereo fundus photographs: Comparison of physician and lay readers. Br. J. Ophthalmol. 61: 192-201, 1977.

Yuen, K.K.: Robustness of some sequential procedures. Commun. Statist. Theor. Meth. A6(1): 43-54, 1977

Schwartz, J.T.: Methodologic differences and measurements of cup-disc ratio. An epidemiologic assessment. Arch. Ophthalmol. 94: 1101-1105, 1976.

OFFICE OF PROGRAM PLANNING AND SCIENTIFIC REPORTING

ANNUAL REPORT
NATIONAL EYE INSTITUTE
July 1, 1976 - September 30, 1977

REPORT OF THE CHIEF, OFFICE OF PROGRAM PLANNING AND SCIENTIFIC REPORTING
Julian M. Morris

Program Planning

The Office of Program Planning and Scientific Reporting continued to provide staff support to the National Advisory Eye Council in regard to the Council's second major planning report, Vision Research--A Five Year Plan, which is due to be published this fall. This report, which will incorporate the work of over 150 consultants, will include a summary volume, a volume which will present reports for each of the five NEI programs plus a discussion on vision research training, and a volume of relevant data which was used by the Council and consultants to determine today's research needs and opportunities in vision science and ophthalmology.

Again this year the Office coordinated the publication of the NEI Annual Report and collaborated with personnel from other offices within the Institute to produce several evaluative and planning documents. The Office was primarily responsible for developing and writing the year's Forward Plan and Evaluation Plan. The Office responded to Congressional and Departmental requests relating to NEI planning activities as well as to requests from other Institutes of NIH for advice regarding program planning activities for use in developing their own research planning. The Office also responded to requests for planning information from organizations and interested members of the general public. The Office also prepared speeches concerning program planning and evaluation for the Director, NIH, and the Director, NEI, for presentations to Congress, higher levels of DHEW, and several national associations related to research in vision and ophthalmology.

Scientific Reporting

The Office continued to give major attention to increased consumer education activities through cooperation with the news media, liaison with agencies serving the blind and visually handicapped, and direct correspondence with consumers seeking a wide variety of information about visual disorders.

Scientific Communications

The Office contributed three articles for the NIH annual publication, Research Advances 1977, which highlights major research developments supported and conducted by NIH during the past year. Research Advances, which is expected to be published in late fall 1977, is distributed to medical schools, grantee institutions, and the public. The Office also contributed an article

on the Diabetic Retinopathy Study to "From the NIH," a monthly column in the Journal of the American Medical Association which summarizes clinically significant NIH research. In addition to disseminating information about scientific meetings, seminars, and workshops conducted by NEI staff, the Office also conducted tours of Institute facilities and program briefings for visiting physicians, scientists, diplomats, four members of the All-Russia Association for the Blind, and several groups of 4-H volunteer leaders.

Consumer Education

The Office continued to expand its activities in consumer education by taking the initiative in a number of projects that are designed to help consumers find information about eye care and visual disorders quickly and conveniently. Among the most practical of these projects is the completion of final drafts for a major HEW consumer publication, Facts About Eye Care and Eye Glasses. Copies of these drafts have been sent to 20 experts throughout the country for review, and it is hoped that the booklet will be published by late fall 1977. The interest and demand for this material is so great that sections of the final drafts have been used as background information for journalists preparing articles on eye care. Five columns were written for Search for Health, which is distributed by the NIH Office of Communications to weekly newspapers across the country. The Office also prepared two radio spots: an interview with Donald Bergsma, M.D., of the NEI Clinical Branch, on child eye care, and a public service announcement offering information on cataract.

Press Relations

The Office responded to written and telephone inquiries from more than 100 publications this year including Better Homes and Gardens, Nieuwe Revu of Amsterdam, Holland, New York Times, Newsday, Reader's Digest, and U.S. News and World Report. A number of writers and editors publications asked the Office to review manuscripts for accuracy prior to publication, an exercise that often helped to clarify information for the writers and editors and their readers. The Office issued nine press releases or announcements, including a major release on the Framingham Eye Study, and prepared 13 articles for the NIH Record. In addition, three articles were prepared for News and Features from NIH, which is distributed to approximately 500 science writers in the medical and general press. The Office has also received over 100 letters since the NEI was listed as a source of information in a Family Circle magazine article concerning where to find the best sources of health care.

Photographs provided by the Office for the Better Vision Institute's New York Times supplement, "Facts You Should Know About Your Vision," led to several hundred requests from doctors, newspapers, low vision clinics, and popular magazines for information about eye diseases and copies of these pictures. The photographs showed how the field of vision may appear to persons with the most common eye diseases. Requests for photographs to illustrate newspaper and magazine articles, textbooks, encyclopedias, and

other specialized publications have continued to increase. This demand had led to expanded efforts to locate new sources of photographs which present a different perspective of eye problems.

Public Inquiries

Responses to telephone inquiries and letters from the public continued to occupy a large portion of time and effort of the Office staff. Approximately 900 letters requiring detailed, reliable scientific information and over 3,200 telephone calls of a similar nature were received during the year. Inquiries concerning cataract, diabetic retinopathy, glaucoma, and macular degeneration were the most frequent requests. The number of letters fluctuated with the number of articles about eye care and NEI in the popular press and was an indication of the effectiveness of our consumer education activities.

The Office responded to 26 Congressional letters and other controlled correspondence and to 32 Congressional telephone inquiries. A growing number of inquiries are also being received from other government agencies; business, professional, and scientific organizations; and publishers of encyclopedias and textbooks concerning statistics on eye disorders, facts on treatment, and current vision research efforts. In addition, the Office provided exhibits on the Diabetic Retinopathy Study for health fairs at a junior college in Baltimore, Maryland, and a hospital in San Jose, California.

Publications

The Office distributed the following number of publications during the year:

Cataract	2,100
Retinitis Pigmentosa	300
Refractive Errors	300
Diabetic Retinopathy	1,020
Retinal Detachment	300
Corneal Diseases	300
Glaucoma	800
Macular Degeneration	1,020
Statistics on Blindness in the Model Reporting Area, 1969-1970	75
U.S. News and World Report, Interview with Dr. Kupfer	200
Evaluation of the Treatment of Diabetic Retinopathy, A Research Project, Reprint from the Sight-Saving Review	120
Vision Research Program Planning	200
Support for Vision Research	240
Summary and Critique of Available Data on the Prevalence and Economic and Social Costs of Visual Disorders and Disabilities (Westat Report)	70
The Framingham Eye Study	75

Miscellaneous

The Office continued to provide background material for use in the legislative process, including opening statements, testimony, contributions to special reports, and detailed answers on specific questions submitted by members of Congress about visual disorders and treatment.

The Office prepared Presidential proclamations for Save Your Vision Week and White Cane Safety Day. A message was also prepared for the Secretary, HEW, to the American Optometric Association's 80th Annual Congress. The Office also coordinated the Institute's contributions to the NIH Annual Report of International Activities, NIH Scientific Directory and Annual Bibliography, Freedom of Information Act Annual Report to the Congress, and the Scientific Information Exchange of the Smithsonian.

As in previous years, the Office continued its liaison with various voluntary and professional organizations including the National Society for the Prevention of Blindness, Inc., Fight for Sight, Inc., the American Foundation for the Blind, Inc., Research to Prevent Blindness, Inc., the Juvenile Diabetes Foundation, the American Diabetes Association, the American Association of Ophthalmology, the American Optometric Association, and the International Agency for the Prevention of Blindness.

EXTRAMURAL AND COLLABORATIVE PROGRAMS

ANNUAL REPORT
NATIONAL EYE INSTITUTE
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REPORT OF THE ASSOCIATE DIRECTOR FOR EXTRAMURAL AND COLLABORATIVE PROGRAMS
William F. Raub, Ph.D.

Fiscal year 1977 was a time of exciting growth and change for the Extramural and Collaborative Programs of the National Eye Institute. In keeping with the Institute's first priority, a record number (608) of grant awards for investigator-initiated individual research projects were made covering a wealth of scientifically exciting ideas relevant to the prevention, treatment, and cure of diseases and disabilities of the visual system. Moreover, there was a marked expansion of the Institute's efforts to evaluate new treatments for diabetic retinopathy and other disorders through the medium of controlled, randomized, clinical trials and considerable progress was made in refining a set of policies and procedures for center grants and research training awards which can facilitate the rapid qualitative and quantitative maturation of vision research. The following sections highlight some of the issues and accomplishments in the NEI Extramural and Collaborative Programs during FY 1977, as well as identify opportunities for future initiatives.

For FY 1977 the National Eye Institute received an appropriation of $64,000,000--an increase of $14,000,000 (28%) over the previous year's appropriation. Of the $64,000,000, a total of $54,186,000 was allocated to Extramural and Collaborative Program activities in the following categories:

Research Grants	$46,024,000
Research Training Awards	4,640,000
Research Contracts	3,522,000
Total	$54,186,000

This funding level enabled the Institute to sustain the rapid but disciplined growth that its programs have exhibited over the past several years.

The bulk of the budget increase occurred in funds for research grants; an additional $10,716,000 was available in this category in FY 1977 as compared to FY 1976. These funds were distributed among the Institute's five programs as follows:

	Research Dollars (in thousands)		
	FY 76	FY 77	% Growth
Retinal and Choroidal Diseases	$12,726	$17,668	39
Corneal Diseases	6,024	7,595	26
Cataract	3,757	4,567	22
Glaucoma	5,473	6,125	12
Sensory and Motor Disorders of Vision	7,328	10,069	37
Total	$35,308	$46,024	30

The grant application receipt rate was 1 1/2 times that in FY 1976, increasing the workload within the Institute and throughout the review system. The National Advisory Eye Council approval rate, however, was stable during these two fiscal years: 83 percent of grants submitted were approved for funding in both FY 1976 and 1977. The Institute was able to fund 53 percent of all approved applications, a slight decrease over FY 1976. The data are given below.

Grant Application Rate

	Received & Reviewed	Recommended for Approval	Approved & Funded	% Funded of all Approved Applications
FY 1976	336	284	162	57
FY 1977	512	425	225	53
% Change	+52%	+50%	+39%	

The percent funded of all approved applications in the Sensory and Motor Disorders of Vision program was considerably lower than in the other four programs (31%). This is due to the greatly increased receipt of applications in FY 1977 and their subsequent high rate of approval (83 percent). The number of approved applications in the Sensory and Motor Disorders of Vision program was equivalent to that of the Retinal and Choroidal Diseases program, the NEI's largest, yet competing funds available to Sensory and Motor Disorders of Vision were less than half of those available to Retinal and Choroidal Diseases.

The distribution of awards between competing and noncompeting research grant applications was as follows:

	FY 1976 Number of Grants	FY 1977 Number of Grants
Prior Year Commitments	444	453
New Research Awards	99	96
Renewal Awards	72	129
Total	615	678

Once the prior year commitments were taken into account, there was approximately $17 million available for new and competing research grants--the largest amount of "new" money for investigator-initiated vision research ever available in one year in the history of the National Institutes of Health. The 678 grant awards represent twice the number awarded in FY 1970, the first year of the National Eye Institute's existence.

The Institute's research grants are comprised of the following categories:

FY 1977 Research Grants by Mechanism
(Dollars in Thousands)

	Number	Total Awarded
Project Grants (R01,R10,R13)	593	$40,468
Special Visual Science Research Awards (R23)	15	134
Core Center Grants (P30)	14	2,386
Specialized Clinical Research Center Grants (P50)	6	1,521
Research Career Development Awards (K04)	35	1,113
Academic Investigator Awards (K07)	15	402
Total	678	$46,024

The codes in parenthesis in the above table are the symbols used by NIH to differentiate the various types of grant awards. A description of each of these mechanisms can be found in the publication, Support for Vision Research--Interim Report of the National Advisory Eye Council, 1976 (DHEW Publication No. (NIH) 76-1098). It is noteworthy that approximately 88 percent of FY 1977 grant funds are allocated to individual investigator-initiated research projects.

The National Eye Institute complements its research grants with a program of institutional and individual fellowships. The purpose of the program is to equip young investigators with the skills, experiences and insights necessary for them to embark successfully on a career in vision research. The program also strengthens the ties between vision science, especially its clinical aspects, and other disciplines, such as the basic medical sciences, epidemiology, engineering and biomathematics.

A total of $4,640,000 was available for support of vision research training in FY 1977, most of it for the National Research Service Awards (NRSA). The individual NRSA fellowship awards accounted for 27% ($1,225,000) of available training funds. The institutional NRSA training awards accounted for $2,440,000 or 53 percent of the program. The remainder of the funds was allocated to a small number of prior year training commitments, i.e., graduate training grants and the Weinberger fellowships. The awards in these last two categories will be funded until their individual project periods expire.

The National Eye Institute's collaborative research activities, funded through contracts, continue to emphasize cooperative clinical trials for the treatment of diabetic retinopathy. The distribution of contract awards and funds is as follows:

	Number	Total Awarded (in thousands)
Diabetic Retinopathy Study	2	$1,035
Diabetic Retinopathy Vitrectomy Study	15	875
Diabetic Retinopathy Study II	20	1,412
Other	1	200
Total	38	$3,522

The qualitative and quantitative growth in the NEI Extramural and Collaborative Programs during the past year were accompanied by several staff and organizational changes:

1. Dr. Thomas C. O'Brien joined the NEI to assume the position of Chief, Scientific Programs Branch. He has responsibility for supervising the Extramural Program Directors and for coordinating activities with respect to vision research training awards and center grants. Dr. O'Brien replaced Dr. Wilford L. Nusser who became the Associate Director for the Extramural Program of the National Institute of Environmental Health Sciences.

2. Mr. William M. Doak joined the NEI to become Chief of the newly created Extramural Services Branch. This new Branch is the result of a reorganization of the management activities associated with the NEI Extramural and Collaborative Programs. The Branch is comprised of three components: a Grants Management Section headed by Ms. Anna Marie Perrell, Grants Management Officer; a new Contracts Management Section temporarily headed by Mr. Doak; and a Management Information Unit headed by Ms. Carolyn G. McHale. Mr. Richard Gruber, the previous coordinator of NEI research contract activities, left the NEI to become self-employed.

3. Dr. Israel A. Goldberg joined the NEI to assume the newly created position of Review and Special Projects Officer. In this capacity he serves as Executive Secretary of the Vision Research Program Committee and coordinates the initial review of center grant applications and requests for academic investigator awards and institutional research fellowships.

4. Dr. Ralph Helmsen left the NEI's Laboratory of Vision Research to become Extramural Program Director for Glaucoma. He replaced Dr. Samuel C. Rawlings who joined the Department of Ophthalmology at the University of Texas in San Antonio.

5. Dr. Herbert Yellin joined the NEI as Extramural Program Director for Cataract.

6. Dr. Thelma N. Fisher joined the NEI as Extramural Program Director for Corneal Diseases.

7. Dr. Luigi Giacometti, formerly Extramural Program Director for Corneal Diseases and Cataract, left the NEI to become Executive Secretary of the Visual Sciences B Study Section, Division of Research Grants, NIH.

VISION RESEARCH TRAINING

The National Eye Institute has long recognized that research productivity depends not simply on the number of investigators but also on their quality and that of the environment in which they work. Therefore, within the limits of legislated and executive authority, the NEI has worked to provide opportunities for the training of individuals in the research laboratories of established investigators and to promote the development and maintenance of centers of vision research that use multidiscipline approaches in the investigation of serious eye and visual system problems. The primary mechanism for support of vision research training is the National Research Service Awards (NRSA) for individual and institutional fellowships. The present status of the NEI's research training activities is given in the table below:

RESEARCH TRAINING a/

	Active Programs	Total Amount (thousands)
NRSA Individual (F32)	104 b/	$1,225
NRSA Institutional (T32)	42 c/ d/	$2,440

Individual NRSA fellowships are directed toward providing training opportunities in all five research programs of the National Eye Institute - Retinal and Choroidal Diseases, Corneal Diseases, Cataract, Glaucoma, and Sensory and Motor Disorders of Vision. Within this framework, research sponsors for trainees are widely distributed, with over 50% of the training provided in non-clinical academic departments. The results from this program have been rather encouraging in that a number of individuals, trained at the postdoctoral level through the Individual Postdoctoral Fellowship and having satisfied the NRSA payback provision requirement, have gone on to receive additional support for their research careers as independent investigators through other types of National Eye Institute support such as the Academic Investigator Award and the individual research project grant.

The intent of the Institutional NRSA fellowships is to encourage work at both the pre- and postdoctoral levels in selected priority disciplines relevant to all five NEI programs as determined by the National Advisory Eye Council. At present, the areas supported include immunology and genetics; pharmacology, epidemiology, physiology and biochemistry; developmental biology, psychophysics and physiologic optics; and pathology. There is also a

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a/ Total obligations for research training in FY 1977 = $4.64 million. In addition to NRSA's, this includes $51,000 for 6 Weinberger Fellowships (F22) and $924,000 for 12 Graduate Research Training Grants (T01). All Graduate Research Training Grants (T01) will be phased out by June 1979.

b/ Includes 42 new F32's

c/ Includes 9 new T32's

d/ 25 program directors on currently active T32's were formerly program directors on Graduate Research Training Grants (T01).

special interest in supporting institutional training programs that will increase the number of clinical investigators who are competent in epidemiology and biostatistics. Recent awards in these areas support a training program in the epidemiology of external eye diseases at the University of Washington and a training program at Tufts University in collaboration with the Harvard University School of Public Health involving epidemiological and biostatistical considerations in vision research and clinical disorders.

At the present time, most of the Institutional National Research Service Awards are made to departments or groups involved in laboratory research. Some of the research training opportunities afforded by these departments or groups include training programs that place emphasis on neurophysiology of the visual system, biochemistry of lens development, microbiology and immunology of anterior segment diseases, pathology and pathophysiology of retinal diseases, biochemical genetics of hereditary retinal diseases, and physiology and pharmacology of the anterior segment. Vision research benefits in a number of ways from the interest that is generated in departments or groups engaged in the above-noted areas of research. First, the number of investigators who are thoroughly trained in a particular area of vision research is increased. Second, the discipline-oriented environment may suggest concepts and approaches that are less likely to evolve in a clinical setting. Third, predoctoral programs of graduate schools are conducted in basic science departments, and the research interests of future investigators are frequently determined at this point.

Institutional National Research Awards that are administered by clinical departments with a complement of established investigators are also useful in providing particular types of research training, especially research training opportunities in studies of clinicopathological correlations in ocular diseases. Examples of such training opportunities include programs at Yale University, the Armed Forces Institute of Pathology (Washington, D.C.), the University of Illinois, and the Eye Research Institute of Retina Foundation (Boston). For individuals with a Ph.D. degree who are interested in vision research, the clinical setting offers an excellent base in which to become acquainted with a broad multidiscipline field. The National Eye Institute has recently awarded a NRSA institutional grant to the University of Florida for a multidiscipline, multi-institutional research training program on the clinical application of psychophysical and physiological optics techniques. This unique program will attempt to bridge the gap between laboratory research findings and their clinical application. This program is a highly specialized training opportunity and responds directly to last year's National Eye Institute workshop on this subject.

The research training programs of the National Eye Institute have had a profound effect on the nation's capacity to conduct research on vision and its disorders. Not only has the number of qualified, independent investigators and organized units actively engaged in vision research increased, but also departments and sections of ophthalmology, schools and colleges of optometry, and other health centers throughout the United States have developed or strengthened their research components so that multidiscipline groups exist and are in a position to address the research problems identified in Vision Research--A National Plan, the forthcoming new report of the National Advisory Eye Council.

CLINICAL TRIALS AND THE TRANSLATION OF RESEARCH RESULTS INTO APPLICATION

Prospective, controlled evaluations of new eye care procedures are an important and growing activity of the National Eye Institute. Studies in this category usually involve random assignment of patients, masked collection and interpretation of data, and specialized biostatistical planning and data analysis. To date, most clinical studies of this sort in the vision area have required the cooperation of several clinical centers in order to acquire a patient population large enough to allow meaningful statistical analysis of the results. These cooperative clinical trials have been supported by the National Eye Institute primarily via the research contract mechanism. Major NEI supported cooperative clinical trials include the trial of photocoagulation for treating proliferative diabetic retinopathy (the Diabetic Retinopathy Study) and the trial of early versus deferred vitrectomy in diabetic patients with advanced stages of the retinopathy (the Diabetic Retinopathy Vitrectomy Study). The importance of these kinds of studies and their relevance to improved eye care is well-illustrated by the article "Preliminary Report on Effects of Photocoagulation Therapy" (Am. J. of Ophthal. 8:383-396, 1976).

Because the day-to-day coordination of such large-scale, cooperative clinical studies requires an extensive commitment of senior scientific and managerial personnel, the National Eye Institute proceeds in a deliberate manner in identifying those eye care procedures that should be evaluated by this approach. At the same time, the Institute recognizes that other extramural support mechanisms need to be developed or extended in order to insure that all meritorious ideas for clinical trials, whether they involve single or multiple centers or whether they originate with Institute staff or the extramural community, have a reasonable chance of being undertaken. Accordingly, beginning late in FY 1976 and extending through the first year, the Institute staff has developed a special set of grant procedures that facilitate the planning and execution of investigator-initiated, controlled clinical trials.

In essence, research grant applicants who request support to carry out a clinical trial either under the individual project grant mechanism (R01) or the cooperative clinical research grant mechanism (R10), and whose ideas and proposed approaches are judged meritorious by an appropriate NIH Study Section and the National Advisory Eye Council, may be awarded funds initially for only the first phase of the work--the development of detailed manual of operations to guide the actual execution of the clinical trial. When such an award is made, future years of support for the conduct of the trial are then contingent upon (1) approval of the manual of operations following a review by expert consultants and Institute staff and (2) the availability of funds.

The decision to take this approach was based upon several factors: (1) the apparent lack of appreciation by many clinical investigators as to what constitutes a well-designed controlled clinical trial (need for consideration of such issues as randomization, stratification of the patient population, inclusion-exclusion criteria, data management and analysis systems, patient follow-up, data and safety monitoring, formalized informed consent procedure, etc.); (2) the National Eye Institute's leadership within the NIH, as exemplified by the Diabetic Retinopathy Study, in the use of well-controlled

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clinical trials (National Eye Institute Workshops for Ophthalmologists, November 6, 1976 -- The Randomized Controlled Clinical Trial, Am. J. of Ophthal. 79: 752-789, 1975; NEI Biometry Branch presentation at the annual course on "Methods of Clinical Research", AAOO Meetings; Ederer, F. Practical Problems in Collaborative Clinical Trials, Am. J. of Epidemiol. 102: 111-118, 1975; NEI Biometry Branch presentations on "Quality Assurance of Clinical Data" at NIH National Conference on Clinical Trials Methodology, October, 1977; Kupfer, C. Clinical Trials, Invest. Ophthal. 15: 513-514, 1976); and (3) the increasing appreciation throughout NIH of the importance of well-developed and carefully controlled clinical trails (Issue paper - "NIH Support of Clinical Trials", Dr. Donald Fredrickson, Director, NIH, August 27, 1975; NIH Conference on Clinical Trials Methodology, October, 1977). Thus, the Institute has seized an opportunity not only to stimulate strong clinical research but also to highlight the ways in which the fruits of vision research, both laboratory and clinical, can be used to improve eye care.

Currently, the National Eye Institute is supporting 10 projects under research grants which involve the development of a manual of operations. The topics of these clinical trials include studies of the effect of vitamin E on retrolental fibroplasia in infants; evaluations of laser treatment for branch vein occlusion, central serous chorioretinopathy, and glaucoma (trabeculotomy); a study of the effects of intraocular lenses on the corneal endothelium; and an assessment of the effect of vitamin C on corneal treatment for acute alkali burns. One clinical trial comparing conventional treatment of refractive errors with orthokeratological treatment has already completed the development of a manual of operations and is about to begin enrolling patients. In another case, the principal investigator determined in the course of developing the manual of operations for a study to determine the efficacy of laser therapy in the treatment of branch vein occlusion that a single clinic study would not provide a sufficient population base and therefore began to develop plans for a multiclinic study using the cooperative clinical research grant (R10). A coordinated set of research grants proposing to employ the manual of operations developed under the initial project grant award has been submitted by the collaborating institutions and is now being reviewed by the Vision Research Program Committee.

At this stage it is too early to measure the success of this approach to achieving well-designed controlled clinical trials or even to ascertain whether it is the best one. But it is clear already that the research community is more cognizant of the importance of this kind of research and is responding well to the Institute's administrative initiatives. The further nurturing of these types of studies will be high on the NEI's list of objectives for FY 1978.

VISION RESEARCH CENTERS: THE NATIONAL EYE INSTITUTE CONCEPT

Until this year, the National Eye Institute has employed three types of large-grants, the Research Program Project Grant, the Core Research Center Grant, and the Specialized Clinical Research Center Grant. In contrast to the regular Research Project Grant, which provides support for a discrete, specified, circumscribed project performed by a principal investigator and his or her own research team, these grants usually provide support for broad-based, long-term programs of research. Generally, they are designed to bring together, through common support, the activities of a number of investigators and teams. However, the ways in which they do this, and the specific purposes of each, differ considerably.

In 1973, the National Advisory Eye Council evaluated the three large-grant types. The Council recommended that the National Eye Institute discontinue use of Research Program Project Grants. Although these awards provided support for a number of projects relating to a common vision-research theme, they also supported projects of mixed programmatic interest to the NEI. In fiscal year 1977, the last Research Program Project Grants supported by the NEI were phased out.

At present, the NEI has a small program of research centers. During FY 1977, there were 13 Core Research Centers receiving NEI support, and six Specialized Clinical Research Centers. Total center support was approximately $3.3 million, or 6 percent of the funds available for the support of extramural research.

In FY 1977, the NEI staff began an evaluation of the two centers programs. As a result, new administrative guidelines for both programs are being prepared. The purpose of this activity has not been to expand the number of centers or to enlarge their scope, but rather to employ these programs optimally in achieving the mission of the Institute while assuring that the mainstay support for vision research, the individual Research Project Grant program, is not jeopardized. In this regard, it should be noted that it is strongly recommended that the NEI extramural staff be consulted prior to the initiation of applications for either program. The Institute's staff is committed to providing guidance to potential applicants, as well as to interacting with ongoing centers in order to assure that the research goals of the Institute are met by the submission of high-quality applications.

Core Research Centers

The Core Research Center Grant does not provide direct funding for research projects. Rather, it provides a central nucleus or core of resources, facilities, and services which are shared by the investigators on a number of individual research projects. Thus, an NEI Core Research Center is an organization at which there are at least four ongoing, high-quality, independently-supported research projects in the visual sciences. At an NEI Core Research Center, investigators are brought together in an environment which facilitates multidiscipline research approaches to problems in the visual sciences. It is an environment which supports laboratory studies, clinical studies,

or both, and which promotes interaction and collaboration among vision researchers and investigators from other academic departments or areas of interest.

The primary objective of the Core Center Program is to achieve research advances which would not be possible through the activities of individual project grants alone. For this purpose, the grant may also provide limited funds to initiate pilot studies which result from the collaborative activities within the Center. In order to get maximum returns from its investment in these highly nutritive research environments, the NEI may also provide annual salary support for newly-recruited investigators whose expertise complements that of staff already on board and who, it is hoped, will eventually compete successfully for their own research project grants.

In fiscal year 1977, the NEI supported ongoing Core Research Centers at the University of Washington, University of Pennsylvania, Eye Research Institute of Retina Foundation (Boston), University of California Los Angeles, University of California San Francisco, Columbia University, Massachusetts Eye and Ear Infirmary, Yale University, University of Rochester, and Smith-Kettlewell Institute of Visual Sciences (San Francisco). In addition three new Core Research Center awards were made to the Medical College of Wisconsin, Mount Sinai School of Medicine, and Harvard University.

Specialized Clinical Research Centers

The Specialized Clinical Research Center Grant is an award which may provide support for both individual clinical research projects and for core resources. The NEI utilizes this funding mechanism only under exceptional, clearly-defined circumstances. A Specialized Clinical Research Center is viewed as a unique environment in which important and definitive clinical research is conducted on a particular human eye problem. The emphasis at these centers is on investigations involving human patients. Most often, the investigations exploit recent laboratory findings in scientific projects involving outpatients. The types of projects conducted, as a rule, would not be practical or possible to pursue outside of the Center. Thus, Specialized Clinical Research Center grants provide support for clinical studies which have a common conceptual framework relative to the etiology, pathogenesis, diagnosis, and treatment of human visual disorders, and which share common practical requirements, such as a unique cohort of patients and special facilities dedicated to clinical research.

Four previously supported NEI Specialized Clinical Research Centers were continued in fiscal year 1977. Each one deals with specific visual disorders: (1) Macular Disease Center, University of Miami; (2) External Ocular Disease Center, University of Florida; (3) Retinal and Choroidal Disease Research Center, University of Chicago; and (4) Glaucoma Clinical Research Center, Washington University, St. Louis. In addition, new centers for Studies of Retinitis Pigmentosa and Allied Diseases, at the Berman-Gund Laboratory of the Massachusetts Eye and Ear Infirmary, and for Study and Treatment of Immune Ocular Diseases, at the Eye and Ear Hospital of Pittsburgh were established.

Specialized Clinical Research Centers provide a superb vehicle for the adaptation of laboratory research findings and techniques to the clinical research setting. As such, this centers program plays a most important role in the execution of the NEI mission. Such centers, with their clinical, scientific, and administrative resources focused on research involving patients in a health care environment, also provide an ideal setting for investigating the translation of the results of laboratory and clinical research to the improved prevention, diagnosis, and management of eye diseases.

RETINAL AND CHOROIDAL DISEASES

Introduction

The retina (the light-sensitive tissue at the back of the eye) and the choroid (an underlying layer rich in blood vessels which is responsible for nourishment of the retina) are the primary sensory and vascular tissues of the eye and are closely related. The optical structures of the human eye focus light on the fovea centralis, a distinct area of the retina which is located temporal to the optic disc. This area possesses the greatest acuity of any portion of the retina by virtue of its high concentration of cone (day-light) photoreceptors.

The region surrounding the fovea centralis, known as the macula lutea or macular region, is normally darker in color than the rest of the retina because it contains a yellow pigment. The macula has the greatest acuity of any portion of the retina and is used exclusively for most visual tasks except for vision in very dimly lighted surroundings. Minute lesions which might not cause visual disturbance in peripheral areas of the retina do cause serious loss of central visual acuity and color discrimination when located in the macular region.

The fovea appears to be directly involved in a variety of retinal degenerative conditions for it is subject to all the pathological alterations which may develop in other areas of the retina. Retinal and choroidal diseases are considered to be among the most difficult visual disorders to manage and require a considerable research effort if a better understanding of their causes is to be gained and more effective approaches to their treatment and prevention are to be designed.

An estimated 15,000 people become blind each year from retinal and choroidal diseases which already account for 42 percent of all blindness in the United States. The causes of blindness from these disorders are diverse and the research relating to them is complex. Therefore, the National Eye Institute's Retinal and Choroidal Diseases program has been divided into twelve subprograms each addressing either a major group of retinal and choroidal diseases or a primary focus of basic research:

Developmental and Hereditary Disorders. These include disorders which afflict the newborn and the young. Many result from known familial tendencies toward blinding disorders.

Diabetic Retinopathy and Other Vascular and Circulatory Abnormalities. Retinal vascular disease associated with diabetes mellitus and the complications of vascular occlusion, leakage of fluids from blood vessels, hemorrhage and neovascularization.

Myopia. Simple refractive errors as well as the pathology which arises in the retina and choroid due to severe progressive myopia which causes the outer coats of the eyeball to stretch and thin. Eventual blindness may result from vitreous changes, retinal detachment and insufficient blood circulation.

Tumors. Malignancies, usually retinoblastoma or melanoma, which may arise from the retina or may result from a metastasis of tumors in other areas of the eye or body.

Macular Diseases. Abnormalities associated with the macular region of the retina frequently associated with aging which cause debilitating loss of central visual acuity.

Retinal Detachment. Separation of the neural retina from the pigment epithelium with a resulting impairment of vision.

Inflammatory Disorders. A group of destructive diseases which can affect all parts of the eye. The causative agents are both infectious and non-infectious and may arise from immunologic insults to eye tissues.

Uveal Tract Disorders. Diseases affecting the choroid, ciliary body and iris. Malfunction of the uveal tract can result in circulatory disorders and the ensuing complications of macular degeneration and inflammation.

Vitreous Humor. An internal structure of the eye which increasingly appears to have a major physiological function as well as a supporting role. Changes in the vitreous humor are associated with aging and with disease processes in such disorders as diabetes mellitus, intraocular infections and uveitis.

Visual Cells and Pigment Epithelium. The specialized cellular elements involved in photoreception. The retinal pigment epithelium is involved in the nutrition of the sensory retina.

Retinal Organization and Visual Adaptation. Information received by the photoreceptor is transmitted through a neural network of four different types of nerve cells for processing within the retina. Failure of this complex organization can result in dysfunctions of perception.

Special Areas of Future Interest. This area includes toxic and environmental disorders, low vision, and retinal regeneration and transplantation.

Vitamin A Deficiency

Krill[1] discussed disorders which cause abnormal night vision and are associated with severe vitamin A deficiency. Vitamin A is of particular interest because of its importance in the visual cycle. Although dietary deficiency of vitamin A is rare in the United States, it may be part of the spectrum of malnutrition associated with alcoholism, drug addiction, and some improperly managed pregnancies.

Deficient absorption from the gastrointestinal tract, faulty liver metabolism and excessive urinary excretion of vitamin A due to systemic disorders are the more likely primary causes of a vitamin A deficiency, as opposed to problems of deficient intake. It is also known that vitamin A in excessive quantities may induce ocular malformations during early stages of embryonic development. To complicate the problem, individual needs for vitamin A vary

widely with no obvious correlations with physical characteristics.[2]

Retinol, a derivative of vitamin A, is bound to the protein of the visual pigment and provides the light-catching ability of the photoreceptors. It is important to describe the routes and mechanisms by which retinol is delivered to the retinal pigment epithelium and photoreceptors.

Supporting evidence for the binding protein for vitamin A derivatives in foveal retinal tissue has been presented by Futterman and associates.[3] Their objectives have been to characterize the reactions of vitamin A associated with light and dark adaptation in the retina and to discover whether vitamin A binding protein exists in human retinal tissue. In addition they sought a noninvasive means of obtaining binding proteins for vitamin A derivatives. The investigators did not find vitamin A binding protein in human fibroblasts but did find retinol and retinoic acid binding proteins in human retinal tissue.[4] The quantity of bound retinoic acid in human tissue greatly exceeds that of retinol. The binding protein for retinol is also present and is more complex in its properties. Nevertheless, these investigators did identify a binding protein capable of interacting with the aldehyde form of vitamin A.[5] These studies indicate the existence of a new binding protein which may be important in the regeneration of bleached visual pigment in the retina.

It now appears that there are intracellular binding proteins for retinol, retinal and retinoic acid. The distribution and function of these proteins within the retina and retinal pigment epithelium in health and disease should be determined. The methodology required for this determination would involve immunochemical, cytochemical and biochemical techniques. The involvement of these intracellular binding proteins in the uptake, intracellular transport, and metabolism of vitamin A by retina and retinal pigment epithelium is important in addition to their potential significance in the delivery of serum retinol and in the transformation of vitamin A in the visual cycle. Pathological similarities between vitamin A deficiencies and retinal degeneration have stimulated investigation of vitamin A uptake and metabolism in retinitis pigmentosa animal models as well as in patients with this and related disorders. The processes that can now be measured are unaffected; however, as more information about the physiological events are uncovered, opportunities to look for specific defects in retinal dystrophies will improve.[6,7]

Vitamin E Deficiency and Retrolental Fibroplasia

Dratz and associates[8,9] are investigating the possibility that some ocular disorders may be the result of deficiencies in one or more antioxidant mechanisms combined with a variety of oxidative stresses. Photoreceptor membranes are susceptible to oxidative damage which may correlate with their high content of polyunsaturated fatty acids. Vitamin E deficient monkeys fed highly unsaturated fat develop clinical symptoms of vitamin E deficiency anemia as well as macular degeneration. Hayes and associates[10] are currently depleting kittens of vitamin A and E in order to evaluate functional and morphological changes in a species with a rod-cone retina. The work may demonstrate that the cat requires dietary supplements of taurine, vitamins A and E, and zinc when fed a casein diet.[11] This model of photoreceptor degeneration is of

particular interest because in the early stages of taurine deficiency in the cat delays occur in the time interval between stimulus onset and the peak of the major cornea-positive component of the cone ERG response (b-wave implicit time). The phenomenon is similar to delays in cone b-wave implicit time seen in some patients with early stages of retinitis pigmentosa.[12]

Studies suggest that although cases of adult human vitamin E deficiency are rare, ocular tissues may be more sentitive to such deficiency than other organs. The development of ocular lesions as a result of dietary antioxidant deficiencies in adult humans may involve marginal deficiencies in dietary unsaturated fats over a long period of time. Thus some senile macular degenerations of the human retina may be related to marginal intake of vitamin E and therefore could be considered deficiency disorders. The protective action of vitamin E in the rapidly developing retinal tissues of the premature infant[13] and kitten[14] is more dramatic than that of the speculated action on the mature human eye.

Kittens exhibit a widely accepted model of the human disease retrolental fibroplasia (RLF). The kitten develops the vasoconstrictive and proliferative changes of RLF seen in the human premature infant; however, the disease in the kitten does not progress through the cicatricial stages which can lead to retinal detachment and blindness. For the premature human infant weighing less than 1,000 grams it appears that any concentration of oxygen in excess of that in air is associated with the risk of developing RLF. To discourage the use of oxygen in the premature infant with respiratory distress, however, is to risk the development of cerebral palsy due to hypoxia.

In order to widen the margin of safety between RLF and cerebral palsy, Boggs and associates[15] and Phelps and associates[16] are developing prospective, randomized clinical trials. Such studies are particularly important in view of the increasing survival of premature infants of less than 1,500 gram birth weight and their relatively high susceptibility of RLF in spite of improved technology and heightened awareness of the need to control arterial oxygen levels. It is possible that vitamin E has the ability to restore physiologic-metabolic balance in rapidly developing tissues and to reverse the tendency to abnormal development in response to the toxic stresses of oxygen and light in the extrauterine environment.

Factors Involved in Retinal Neovascularization

Neovascularization is a retinal response which occurs in proliferative retinal diseases such as diabetic retinopathy, sickle cell retinopathy, retrolental fibroplasia and retinal venous occlusive disorders. These disorders are among the major causes of blindness. In these conditions new blood vessels proliferate on the surface of the retina and branch into the vitreous body. The proliferating vessels are fragile and hemorrhage into the vitreous. Eventually fibrous tissue forms which through traction can cause retinal detachment and blindness.

Currently there are no means of preventing or curing proliferative retinopathies, particularly when they are in advanced stages. It is generally

accepted that neovascularization is a tissue response to clinically observable events. In response to a stimulus, focal areas of the retinal capillary bed may lose their perfusion capability, and the retina becomes ischemic. The retinal tissue then becomes deficient in essential nutrients and gases, and metabolic products accumulate. It is hypothesized that ischemic retinal tissue could be a source of a vasoproliferative substance which could diffuse throughout the retina as well as to other vascularized eye tissues.

Chen and associates [17,18] recognize that observations of the physiology, chemistry, and clinical role of vasoproliferative substances depend upon the development of a suitable animal model and bioassay techniques. The problem is specific and can be quantitated. Concurrent with the vasoproliferative studies is an investigation that has as its ultimate goal the diminuation of angiogenesis activity in human retinal vascular disease, particularly in diabetic retinopathy.

Finkelstein and associates[19,20] have been studying the effect of tumor angiogenesis factor on retinal vessels in rhesus monkeys. This factor had been shown to elicit neovascularization in various tissues but its effect on retinal vascularization has not been previously demonstrated. The investigators have shown that a slow release polymer pellet impregnated with tumor angiogenesis factor, when placed on the surface of the optic nerve head, will stimulate disc neovascularization. In this manner, a model for detecting the presence of human proliferative substances may be approached and means of preventing retinal neovascularization considered.

Disorders of the Macular Region

Central Serous Chorioretinopathy. Central serous chorioretinopathy is a sporadic disease of unknown cause which may affect the eyes of otherwise healthy young persons, usually men between the ages of 20 and 40 years. The symptoms consist mainly of loss of central visual acuity in one eye along with image distortion and impairment in visual space perception. Symptoms may also include subjective disturbances in dark adaptation and color vision.

Some clinicians believe that central serous chorioretinopathy poses no great threat to sight because the disorder is self-limiting. However, others take the position that although a slow, spontaneous remission with improvement of visual function generally occurs over a period of months, the recovery time is unpredictable.[22] If recovery is slow or recurrences frequent, the danger of permanent visual loss is increased.[23]

Individuals with central serous retinopathy have what appears to be leakage of serous fluid from a site within the choroidal pool across Bruch's membrane through focal defects in the usually tight intercellular junctions of the retinal pigment epithelium. The serous fluid accumulates under the sensory retina, producing a localized nonrhegmatogenous retinal detachment which compromises retinal function at that site and thereby produces a disturbance in vision. The fluid which has accumulated in the subretinal space may gradually disappear over a period of weeks or months and be accompanied by spontaneous reattachment of the retina to its underlying pigment epithelium. Though the mechanism of fluid disappearance is unknown, the retinal vessels, the pigment

epithelium of the retina, and the choriocapillaris may be involved in the removal of the subretinal fluid. However, very little is known about the physiological factors favoring removal of fluid from the subretinal space.

There is evidence that laser or xenon arc photocoagulation of the retina can increase the rate of fluid disappearance from the subretinal space. Therefore, the National Eye Institute is currently supporting a clinical trial to determine the efficacy of argon laser photocoagulation as a treatment for central serous chorioretinopathy and to learn how such treatment might promote early disappearance of abnormal subretinal fluid.[23]

The use of laser coagulation to improve the treatment of central serous chorioretinopathy will make available to the clinician a technology and possible insight into the dynamics of this disorder.

Senile Macular Degeneration. Of the blinding retinal diseases, senile macular degeneration accounts for at least 20 percent of new blindness which occurs annually. Choroidal neovascular membranes are of importance in senile eyes and play a prominent role in central visual loss in many maculopathies. Some clinicians use photocoagulation treatment for senile maculopathy at various stages.

Past results of therapeutic argon laser photocoagulation have not been sufficiently good to permit meaningful conclusions to be drawn regarding the value of this treatment in the management of the senile macula with disciform detachment.[24] A clinical trial with random assignment of eyes to treatment or nontreatment is necessary to define the role of photocoagulation in this disorder. Therefore, the NEI is supporting a randomized clinical trial to evaluate the role of photocoagulation in the management of choroidal neovascular membranes in the senile macula.[24] Although this study does not seek to discover the cause of senile macular degeneration, it does seek to develop the best possible data on prognosis and to determine whether photocoagulation is effective in the treatment of senile macular degeneration.

Animal Models for Maculopathies. Disciform macular degeneration characterized by subretinal neovascularization in the macula has been produced in stump tail monkeys, by Ryan and coworkers.[25] Once the break in Bruch's membrane had been produced by mechanical and/or chemical means and subretinal neovascularization produced, the pathogenesis of disciform degeneration was studied. The work has shown that it is possible to produce subretinal neovascularization in the monkey. In addition, Ryan and associates have also found three monkeys with naturally occurring drusen which tend to be associated with senile macular degeneration in man.[26] These animals are currently being studied with repeated fluorescein angiography and fundus photography. Eventually, clinicopathological correlation of Bruch's membrane and pigment epithelium will be made.

During the past year, Vainisi and associates have studied three separate families of baboons (16 animals).[27,28] Two of the families are composed of members who have at least one parent affected with macular degeneration. The other family has never had any members affected with macular degeneration and is being used as a control. Each baboon has been studied by fundus photography,

by fluorescein angiography, and by electrophysiologic and biochemical techniques. The affected animals display a type of abnormal behavior that is associated with decreased vision. The evidence from these studies supports the hypothesis that macular dystrophy begins in the cone outer segments and in advanced cases of diffuse dystrophy eventually involves all the photoreceptors.

Continued study of non-human primate models will greatly advance our knowledge of the pathogenesis of macular diseases. It is hoped that such studies will reveal abnormalities responsible for the fundus changes and make possible the design of new approaches to treatment.

Toxic and Environmental Factors

Continuous outer segment disc renewal is a basic property of all vertebrate rod photoreceptors and the disposal of shed old discs involves an intricate cellular interaction between the rods and pigment epithelial cells in maintaining a relatively constant rod outer segment length. The approach to studying the mechanisms involved in this process is based upon observations that light stimulates disc detachment and phagocytosis by the pigment epithelium. By using the experimental manipulation, the disposal mechanism can be studied more accurately because the process can be initiated and then examined with time, instead of having to piece together isolated events without knowing which preceded the other.

The phenomenon and defects in phagocytosis has stimulated investigators to delve into basic mechanisms in animal models with health related implications.[2] Noell and associates are making major efforts to correlate the measured vulnerability of photoreceptors to damaging light with biochemical changes in the rhodopsin, lipids and fatty acids of the rod outer segments.[30, 31] The kinetics of changes in these constituents were determined in long-term adaptation to darkness and weak cyclic light and related to the ability of strong light to cause visual cell death. The latter is measurable by fractionable irreversible ERG loss and by quantitative histology. Noell and associates have concluded that the synthesis or membrane incorporation of rhodopsin, phospholipids and highly unsaturated fatty acids are controlled by environmental light. Maintaining animals in darkness apparently accelerates these processes to the maximum, and a low intensity environmental light depresses the rhodopsin level in relation to phospholipid content. In a highly intense lighted environment, the phospholipid content is first altered, followed by the fatty acid composition. These adaptive changes are being investigated in relation to vitamin deficient diets and ambient temperatures.[30,31]

La Vail and associates have shown that when albino rats are reared in cyclic light, a burst of rod outer segment disc shedding occurs soon after the onset of light[31,32,33] Furthermore, the burst of disc shedding follows a circadian rhythm for at least three days. Further study of this phenomenon in a variety of modifications of the lighting environment is in progress.

Cooperative Clinical Trials

The National Eye Institute is currently pursuing three contract-supported, multicenter, randomized, controlled clinical trials which are titled:

1. Diabetic Retinopathy Study - Phase I (DRS-I)
2. Diabetic Retinopathy Study - Phase II (DRS-II)
3. Diabetic Retinopathy Vitrectomy Study (DRVS)

DRS-I. This study was designed to determine whether photocoagulation is of benefit in preserving vision in patients with proliferative diabetic retinopathy. The study began in 1971, and enrollment ended on September 30, 1975, after 1758 patients had entered the trial. To be eligible for the study, patients had to have proliferative retinopathy in at least one eye or severe non-proliferative retinopathy in both eyes. Visual acuity of 20/100 or better was required in each eye. Both eyes had to be suitable for photocoagulation and neither eye could have received it previously. One eye of each patient was assigned randomly to prompt photocoagulation and the other to follow-up without photocoagulation.

In its first report the study showed treatment to be of substantial benefit in reducing the risk of severe visual loss, defined as visual acuity of less than 5/200 at each of two consecutive four-month follow-up visits.[21] Some harmful effects of treatment were also reported. For eyes with certain characteristics the risk of severe visual loss without treatment was found to be so great and the reduction of this risk with treatment so impressive that they outweighed the risks of the harmful effects of treatment. Accordingly, the Study's protocol was changed to require consideration of photocoagulation treatment for initially untreated eyes with these characteristics.

The groups of previously untreated eyes in which treatment was to be considered were defined as those with:

1. moderate or severe new vessels on the disk (NVD), with or without vitreous or preretinal hemorrhage;

2. mild NVD with vitreous or preretinal hemorrhage; and

3. moderate or severe new vessels elsewhere (NVE) with vitreous or preretinal hemorrhage.

It was concluded that in most eyes with these characteristics, the risk of deferring treatment is substantial and prompt photocoagulation is usually desirable.

Following publication of its first report, the DRS continued follow-up of its patients under a modified protocol allowing, but not requiring, treatment of eyes originally assigned to no treatment if they met these high-risk criteria. Large numbers of patients have been followed for three years and the first fifth year follow-up visit has occurred. The DRS is now concentrating its analyses on determining the effect of photocoagulation on the development and progression in eyes with milder stages of retinopathy than those specifically mentioned in its first publication.

A monograph giving details of study design and procedure and presenting baseline characteristics of patients is being prepared for publication, as is a methodologic paper on fundus photograph grading.

The DRS has entered an analytic phase where emphasis will be placed on developing detailed descriptions of the natural history of diabetic retinopathy and elaborating on the effects of photocoagulation treatment. These will include attempts to identify additional systemic and ocular risk factors that are associated with the progression of proliferative diabetic retinopathy and to determine whether photocoagulation treatment exerts its effect by altering these factors or by an independent route. Because it is not known how photocoagulation exerts its beneficial effect, attempts are being made to develop a pathology study within the DRS. Leading ocular pathologists from around the country have met to develop a protocol for examining eyes of DRS patients.

The DRS research group realizes its obligation beyond publication of a scientifi paper to disseminate its findings broadly to the medical community. The group prepared an exhibit for this purpose which was displayed at the American Academy of Ophthalmology and Otolaryngology meeting in October 1976. The study group plans to continue and expand this type of educational effort and attempts are being made to cooperate with non-DRS investigators in developing consensus for clinical practice.

DRS-II. This study has been designed to answer some of the major questions which were not addressed by the Diabetic Retinopathy Study Phase I. The objective of this new trial is to determine the optimum stage to initiate photocoagulation, the effectiveness of photocoagulation on diabetic maculopathy, and whether aspirin administered at various stages of diabetic retinopathy can prevent or retard the progression of this disease. A working group was established to prepare an outline of the proposed trial. The outline was presented to the National Advisory Eye Council which endorsed it at their January 1977 meeting. The NEI issued a Request for Proposal on April 30, 1977 for potential clinical centers. Proposals that were submitted before the June 15 deadline are in the process of being reviewed, and negotiations and funding are to be completed by September 1977. In addition to the clinical centers, proposals have been solicited for a coordinating center, fundus photograph reading center, and central laboratory facility.

During the next year the study participants and NEI staff will collaborate to complete the study design and develop a detailed manual of operations. Recruitment of patients should begin by September 1978.

DRVS.

This study was initiated to test vitrectomy in the treatment of severe vitreous hemorrhage due to diabetic retinopathy. The Diabetic Retinopathy Study, Phase I and II, is examining the response to photocoagulation treatment of eyes having diabetic retinal vascular disease without extensive intraocular hemorrhage. The DRVS deals with a more advanced stage of this disease in which blindness due to hemorrhage into the vitreous has occurred. Such eyes are not suitable for photocoagulation treatment since the extensive hemorrhage prevents access of the light beam to the retina. Vitrectomy removes the opaque vitreous barrier to vision and reduces the risk of serious secondary mechanical changes in the retina such as traction detachment.

The vitrectomy procedure being examined in the DRVS involves an instrument which combines cutting, suction, and infusion of a replacement solution. The instrument is inserted through a small incision in the side wall of the eye.

In a number of medical centers, vitrectomy for long-standing diabetic vitreous hemorrhage has become a standard procedure. The vision is restored to useful levels in a moderate number of such patients who would have a high probability of remaining blind without vitrectomy. However, the vision does not always improve following vitrectomy in eyes with long-standing vitreous hemorrhage. Inoperable retinal detachments or other sequelae of diabetic vitreo-retinal disease may occur during the conventional one-year waiting period following vitreous hemorrhage. In other eyes surgical complications of the vitrectomy procedure itself make restoration of vision impossible. Some vitreous surgeons believe that the operation should be done earlier in the period following severe vitreous hemorrhage. They hope that by performing surgery earlier, (within the first six months of severe hemorrhage), the development of inoperable sequelae of the hemorrhage will be lessened and the average level of vision restored improved.

However, it is not known whether earlier operation would be as safe as later surgery. To determine this a clinical trial is the most ethical and scientific way of proceeding. Randomization, the most important feature of a clinical trial, tends to make treatment groups similar in all respects except for the treatment assigned. Thus, any observed differences may usually be ascribed to the treatments themselves rather than to differences in patient characteristics that would influence response to surgery.

In the DRVS eyes with severe vitreous hemorrhage (the H Group) are assigned to either vitrectomy within the first six months following the hemorrhage or to a "late" vitrectomy group in which vitrectomy will be performed at one year following hemorrhage in those eyes still suitable for it. A separate category (the N Group) consists of eyes without extensive vitreous hemorrhage that are followed without treatment to collect natural history information. Photocoagulation, based on information learned from the DRS, is given to those eyes in either H or N Group as indicated.

Following the decision to proceed with DRVS, contracts for seven clinical centers, a fundus photograph reading center, and a data coordinating center were awarded in June 1975. Contracts for six additional clinical centers were awarded in June 1976. Recruitment of the first of a projected 826 patients was begun in October 1976 by those centers completing detailed procedures for initiation of data collection required by the protocol.

The rates at which the clinical centers have recruited patients meeting the highly specific DRVS eligibility criteria have been somewhat lower than originally expected. Further attention by investigators to their referral sources has been encouraged, and the National Eye Institute has written to all U.S. ophthalmologists asking for their assistance. An exhibit to encourage patient referral was displayed at the 1976 meeting of the American Academy of Ophthalmology and Otolaryngology and lecture material and printed

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materials have been made available to DRVS investigators for use at other local and national medical meetings. Editorials have also been written describing the DRVS for key medical journals.

In May 1977 the National Eye Institute began a special six-month monitoring period during which the results of the recruitment efforts of each DRVS clinical center will be tracked closely to determine the feasibility of the original Study goals. By June 30, 1977 a total of 114 Group H and 169 Group N had been found eligible for the Study after completion of one baseline visit. Of these, 58 and 65 eyes respectively had been randomized or assigned to a Study group.

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CORNEAL DISEASES

Introduction

Over 2 million cases of corneal disorders and diseases occur each year in the United States. These account for 62 percent of the total incidence of all acute and chronic disorders, diseases, and injuries to the eye. Although the incidence of legal blindness from corneal diseases represents approximately 6 percent of all legal blindness, corneal and external eye disorders and diseases cause severe disability and pain and require a considerable amount of patient care.

The anatomic position of the cornea and adjacent structures contributes much to their susceptibility to ocular infections, allergies, and injuries. Some of the causes of cornea disease include bacterial, fungal, and viral infections, inflammatory reactions, improper moistening and covering of the cornea by the eyelids, birth defects, and degenerative conditions.

The following highlight research areas where successes have been achieved during the past year in addressing certain important problems related to corneal diseases.

Infectious Diseases and Corneal Inflammation

Herpetic infections of the cornea are one of the most prevalent disorders observed in clinical practice. Cavanagh reports that 46 percent (52 of 114 observed) of the corneal disease patients seen in his clinic have ocular herpes infections.[1] This condition is seen three times more frequently than his next highest category, post-surgical healing, which accounts for 13 percent of his patients.

Forrest and Kaufman, in a case report, pointed out that it is not always easy to diagnose viral agents without support of laboratory findings.[2] They encountered a person with a corneal infection resembling herpes zoster which had been described as such by another ophthalmologist, but which turned out to be due to herpes simplex virus, type 1. The first physician treated the patient with topical corticosteroids and atropine with negative results, whereas with proper diagnosis the condition was treated successfully with trifluorothymidine and prednisone.

Some recent studies with herpesviruses provide useful information concerning treatment. Phosphonacetic acid had a significant antiviral effect when applied topically in liquid and ointment preparations on superficial herpetic keratitis in rabbits.[3] It is also effective in the treatment of idoxuridine-resistant herpetic keratitis in rabbits.

In studies of the effects of high doses of adenine arabinoside (Ara-A) on humoral immunity in white rabbits and guinea pigs, no immunosuppressive activity of Ara-A was detected.[4]

In herpes simplex studies carried out in Tunisia,[5] it was found that herpes simplex ulcers of the corneal epithelium that were treated by denuding the

cornea with a cotton-tipped applicator healed more rapidly (2.4 days) that those treated with idoxuridine (7 days).[5] Present studies are directed towards delineating the degree of incapacity and economic loss incurred by patients affected by ocular herpesvirus infections in Tunisia. Subsequent studies are to include evaluation of the antiviral compound trifluorothymidine.

In studies involving herpesvirus infections and latency, virus was recovered from the trigeminal, superior cervical and ciliary ganglia.[6] During acute phases, unilateral herpesvirus inoculation of animals which had previously experienced herpesvirus infections did not lead to acute involvement of these ganglia,although control animals had ganglia infections localized in the inoculated side. Virus was shown to be latent in the ganglia of one rabbit six months after initial infection. These pilot studies have paved the way for future investigators to gather more information regarding the latency and transforming potential of this ubiquitous virus.

Jawetz has prepared a report on antiviral chemotherapy in which he describes present day choices for treatment.[7] Methods were documented for treatment of infection by herpesviruses and other small nucleoprotein-containing agents.

Sery has contributed valuable preliminary information concerning herpes viruses and the immune response.[8] He separated herpes simplex virus and virus-specified proteins and used whole virus and viral specified proteins in testing the immune response of local regional lymph nodes to these antigens via in vitro cell-mediated immunity assays. Then he observed the reactivity of lymph node cells to both T cell mitogens and specific herpesvirus antigens in animals with acute dendritic keratitis and disciform keratitis and found that the two diseased states provided different responses. The lymph node cells of animals with active dendritic lesions reacted well to both T cell mitogens and specific herpes antigens over the first twelve days after infection. In contrast, lymph node cells removed from animals undergoing disciform keratitis reacted very strongly to the phytohemagglutinin but showed little or no response to specific herpesvirus antigens.

Presently, protocols are being extended to include peripheral blood lymphocytes. It is expected that some correlations may be found among responses observed in peripheral blood lymphocytes, the regional lymph nodes, and the state of the disease (e.g. acute dendritic keratitis, recurrent infection, scar formation, etc.). Such correlations, if they exist, would be important because in monitoring the human disease, the easiest access to tissue for evaluation purposes is via blood samples. Sery's observations and the results of further experiments could have very practical applications.

An interesting investigation is underway to assess the cause and prevalence of superficial punctate keratitis and unclassified uveitis in Alabama.[9] Punctate keratitis has an unusually high incidence in Macon County, Alabama; sixty-eight new cases were recorded for this area in 1976. This is a 66 percent increase over that observed in 1975. This community, therefore, provides a particularly good opportunity for classical epidemiological evaluation of a very serious problem.

Settler and his colleagues are carrying out laboratory isolation procedures for the detection of viral agents in the cornea and conjunctiva of Macon County residents afflicted with punctate keratitis. Preparations are being cultivated in human amnion, kidney, and fibroblast tissue cultures. Data from control studies indicate that no viruses exist normally in human aqueous humor. Eventually, this survey will entail serological studies also. If any viruses are isolated, the agents will be transmitted to rabbit eyes for additional characterization.

A major goal of Dawson and colleagues is to evaluate methods of preventing visual loss due to inflammatory eye diseases such as trachoma and acute hemorrhagic conjunctivitis as well as those complications caused by herpesviruses, adenoviruses, pneumococci Haemophilus species, and Staphylococcus aureus. They hope to provide measures for treatment, control, and prevention of such infections. Many of the observations recorded below are a result of conjoint projects conducted in the United States, Tunisia, and Egypt. Following initial field studies, clinical methods for diagnosis and treatment of trachoma were published in the Bulletin of the World Health Organization (WHO). Recently, these procedures were recommended officially by WHO for use in their programs.

In family studies involving both adults and children, Dawson[10], Whitcher[11], et al, found marked reduction in chlamydial organisms in persons treated with 1 percent tetracycline ointment or with the antibiotic delivered in a thin plastic wafer which fitted under the eyelid. As a result of these treatments, they hope to achieve a lower trachoma reinfection rate.

Epidemiological studies of trachoma in Tunisia indicated that when inflammatory eye diseases in children under ten resulted in conjunctival scarring there was a risk of visual loss for the individuals as adults. Inflammatory disease was due to both chlamydia and bacterial pathogens present in the conjunctiva. Kock-Weeks bacillus was also isolated from the eyes of trachomatous children in Egypt and Tunisia. A similar study is underway now to assess the conjunctivitis caused by gonococci; there have been sporadic outbreaks in Egypt.

A study of neonatal conjunctivitis in San Francisco showed that in the first month of life, chlamydia was the most frequent cause.[10] Staphyloccus aureus affected the eye throughout the first two years.

Kuo and Grayson, in a basic tissue culture growth experiment, infected two different tissue culture cell lines with chlamydia and Lymphagranuloma venereum.[12] They measured attachment to and inclusion within the cultured cells in these systems and found that the rate of attachment was temperature-dependent with both agents. Cell susceptibility was important for inclusion formation of trachoma.

Bacterial products have been implicated in pathological processes affecting the cornea. Recently, evidence was presented indicating that a penumococcal cytolysin contributes to the pathogenesis of corneal disease.[13] For some time, investigators have accepted the idea that, in various types of bacterial

infections of the cornea, cytolytic agents activate host degradative enzymes and thereby contribute to the breakdown of corneal tissue. Johnson demonstrated intracellular localization of pneumococcal lysin and recovered extracellular Pseudomonas aeruginosa hemolysin.[13] These studies are continuing with a view toward understanding the genetic basis of lysin production, binding requirements, mode and site of lysin action, and the use of inhibitors for control of degradative processes.

Identification, treatment, and control of fungal agents which can infect the cornea remains a continuing problem. Recently, new successes and new difficulties which warrant attention have been reported. A shortened version of Grocott's methenamine-silver technique for use in staining corneal scrapings was devised, and this procedure was found to be superior to current methods for the diagnosis of mycotic keratitis.[14]

A double infection by Phialophora verrucosa (Medlar) and Cladosporium caladosporioides (Frescenius) was reported.[15] These fungal agents, found infrequently in corneal infections, were isolated from a corneal ulcer. The condition did not respond to antivirals, steroids, and Pimaricin nor to the conjunctival flap procedure. Penetrating keratoplasty was performed with good functional and optical results.

Human transfer factor was administered locally and systemically to guinea pigs with Candida albicans keratitis or those with herpetic keratitis; however, this type of therapy did not alter the course of the disease in these animals.[16]

Rebell and Forster described four cases of human keratitis caused by the tropical fungus, Lasiodiplodia theobromae, which was encountered recently in the Miami, Florida area.[17] (There are only eight known cases in the world). The fungus was found to be endemic in Miami, where it was recovered from home-grown and imported bananas. In vitro polyene antimycotic antibiotics were fungicidal for the agent. When rabbits were inoculated with fungal isolates from these patients, progressive corneal ulcers were produced; these organisms appear to have collagenase activity.

Effects of Drugs, Metabolites, and Hormonal Substances on the Cornea

Pharmacokinetic studies were carried out in the eyes of rabbits and man.[18] Following subconjunctival injections of various tracers, their movements were observed. It was found that the penetration of fluorescein dye into the anterior chamber of the eye, when administered subconjunctivally, was 100 times greater in man than in the rabbit. These observations suggest that drugs which are intended to be administered by subconjunctival injection must be tested this way in man before being accepted for general use.

A new investigation was started at the Edward S. Harkness Eye Institute, Columbia University on the allergic reactions of conjunctival tissue.[19] This project involves a collaboration between Dr. W. J. Manski (ophthalmology) and Dr. K. E. Eakins (pharmacology). Taking advantage of the fact that the conjunctiva is a lymphoid mucous membrane which participates in many diseases

of the reticulo-endothelial and lymphatic systems and which has pronounced effects on immune responses, these investigators observed a powerful synergistic effect of histamine and prostaglandin when applied simultaneously to the conjunctiva. When these drugs were used separately, there was greater vasodilation and edema. Histamine in combination with prostaglandin E induced an inflammatory response associated with severe cellular infiltration of the conjunctiva; this was not found when these drugs were applied separately. Optimal response was obtained in about 45 minutes and persisted one day.

A compound designated 48/80 and known to degranulate mast cells was tested in the rabbit conjunctiva; vasodilation and edema developed. In this case, the response reached its peak in 24 hours. Experiments of longer duration using compound 48/80 are planned in an effort to understand the role of histamine-like agents as mediators of anaphylactic reactions in the eye. It is of interest to note that this cross-discipline collaboration has produced very rewarding data in a short time.

In studies[20-24] involving the kinetics and mechanism of corneal transport of pilocarpine and fluorometholone the rate limiting step for pilocarpine was shown to be in epithelial uptake, whereas for fluorometholone, diffusion through the stroma was rate limiting. The investigators established rate constants for uptake and disposition of these drugs and refined experimental procedures for studying epithelial and conjunctival concentrations of various types of drugs. Also, protein binding studies were carried out with sulfisoxazole.[20] It was shown that this drug attaches to various protein fractions such as alpha globulin, gamma globulin and lysozyme. The data quantitatively accounted for the overall binding of this drug in the protein fractions of human tears.

Baum and associates studied ocular penetration of antibiotics in a model system.[25-26] They also studied concentration effects, interference reactions, and binding of antibiotics to various components such as pigments. They hypothesized that a tight binding of antibiotic to pigment occurred. They found that synthetic melanin (100 to 1,000 ug per ml) inhibited the in vitro activity of aminoglyosides and tetracyclines, but did not affect the activity of beta lactam antibiotics, erythromycin, or clindamycin.

Several innovative projects relating to nutritional and metabolic factors were initiated during the past year. Pfister and associates studied epithelial and stromal repair in alkali-burned rabbit eyes.[27,28] They observed depressed ascorbic acid levels in samples of aqueous humor taken during the period of repair. Following a 20 sec., 12 mm diameter, 1.0 N sodium hydroxide burn, glucose levels in aqueous humor returned to normal in a short time but ascorbic acid levels remained depressed for up to 30 days. Nearly all corneas become ulcerated and corneas in about 60 percent of the animals tested perforated. Following 12 mm alkali burns, rabbits treated daily with 1.5 grams ascorbic acid subcutaneously rarely developed corneal ulcerations and no perforation occurred. It was suggested that exogenous maintenance of adequate levels of ascorbic acid in aqueous humor overcame the relatively scorbutic state of the anterior segment induced by alkali burns and thereby blocked the development of corneal ulceration and perforation. Elevated levels of ascorbic acid in

aqueous humor has no influence on corneal epithelial cell migration patterns following alkali burns. Pfister now is preparing to conduct a clinical trial of topical ascorbic acid therapy in patients who have been victims of corneal burns or who have fungal or herpetic involvement of the cornea to see if it is as effective in humans as it is in the animal model.

In October 1976, a workshop on keratomalacia was conducted by the National Eye Institute.[29] An important objective of the meeting was to spark renewed interest in studies involving the effects of vitamin A deficiency on the cornea. An expansion of research in this area is expected to result from this workshop.

The effects of several agents which increase intracellular levels of cyclic AMP (cAMP) in corneal epithelial cells were studied by Cavanagh. For some time, it has been known that patients who use epinephrine habitually for the management of glaucoma often suffer from recurrent punctate keratitis of the corneal epithelium. Furthermore, previous work showed that increased intracellular levels of cAMP, when mediated by prostaglandins, epinephrine, and nor-epinephrine, cause a profound decrease in cell locomotion and a corresponding increase in cell adhesion; in fact, an increase in the intracellular level of cAMP in many eukaryotic cells was found to be a growth inhibitory signal. Cavanagh and colleagues, in preliminary experiments, added dibutryl cAMP in concentrations ranging between 10^{-2} and 10^{-6} M, with and without theophylline at the same molarity, to epithelial cells cultured in various media.[30] In all cases they observed that all growth activity ceased at levels above 10^{-4} M cAMP and theophylline. They observed the same effect of epithelial regrowth on whole corneal buttons placed in organ culture and on reepithelialization of gently denuded epithelium in rabbit corneas in vivo.

Because clinical inflammation often is associated with persistent lack of healing of epithelium in human patients, they attempted to reduce inflammation in both animals and humans with non-steroid anti-inflammatory substances such as aspirin and indomethacin. These agents are known to inhibit the synthesis of prostaglandins which in turn presumably act to raise intracellular levels of cAMP. Cavanagh's studies indicated that these non-steroid anti-inflammatory agents seem to influence healing of persistent epithelial defects in a positive way in animals; however, the effects are not marked in man. A double-blind clinical evaluation is planned. Fenoprofen will be included in future clinical evaluations.

Functional Aspects of the Corneal Surface, Stroma and Endothelium; Hydration and Transport Mechanisms.

Research in corneal surface chemistry is currently aimed at determining the amino acid composition of proteins found in secretions from meibomian glands. Glough found that proteins comprised about 12 percent of the total dry weight of preparations extracted with benzene and water from secretions expressed from 150 eyelids. Following polyacrylamide gel electrophoresis, six protein bands were obtained and molecular weights were estimated. The fast moving band had the same mobility as myoglobin. Preliminary amino acid analyses of total proteins indicated that glycine and serine were present in amounts higher

than those generally found for the composition of proteins. A careful analysis of all protein bands is underway.

In the cornea, fibrils scatter light and transparency results from interference effects due to an ordering in the spatial arrangement of the fibrils about one another. Farrell is interested in this ordering and its disruption in abnormal corneas.[32] By light scattering and electron microscopic evidence, he shows that during swelling there are regions void of fibrils which result in the formation of so called "lakes", and this condition alters light transmission. An apparatus recently developed by Kim provides a procedure for measuring light scattering from the cornea at small scattering angles which is very useful in testing the above hypotheses.[33]

Pseudomonas protease elaboration studies and their collagenase activities on ocular tissues are underway. Kreger purified various pseudomonas proteases and found that they had an isoelectric point of approximately seven and a mol. wt. of approximately 20,000 (as determined by gel filtration).[34] He plans to assess the effects of pseudomonas proteases on the integrity of corneal cells maintained in tissue culture. Currently, Kiorpes and associates are testing the possibility that destruction of the cornea may result from advanced vitamin A deficiency because of the inability of the system to control collagenase.[35] They show that alpha-1- macroglobulin inhibits collagenase liberated from ulcerating (alkali-burned) corneas maintained in organ culture. Also, they show that in severely vitamin A-deficient rat corneas maintained in culture, both collagenase and hydroxyproline are released. The latter is a product of the enzymes' action on the corneal stroma. Experiments are in progress to attempt to show that "corneal melting", which occurs in vitamin A deficiency, is related to the decline in serum alpha-1-macroglobulin.

Dibutyryl cyclic AMP (D.B. cAMP) and theophylline partially inhibit the appearance of collagenase activity when added to cultures of ulcerating rabbit corneas.[36] Drug treatment with D.B. cAMP and its hydrolysis product 5 AMP both suppress collagenase activity and inhibit the degradation of explant collagen. The exact mechanism by which these drugs act is not known, but it is under study. It may be that these drugs affect the synthesis and/or the secretion of collagenase. Berman and associates suggest that "first messengers" exist which can prevent the secretion of corneal collagenase by raising the endogenous cAMP level in corneal cells through stimulation of the adenyl cyclase system.[36] They suggest further that treatment of corneal ulceration may require intervention at several levels such as biosynthesis, secretion, activation and collagenase activity.

The corneal endothelium has undergone intense investigation over the past year. Use of the specular microscope has contributed significantly to the success story.[37] Bourne and Kaufman, using a modified clinical specular microscope at high magnification in routine examinations of forty patients, were able to detect endothelial damage or disease that was not seen by slit-lamp examination.[38] By means of the specular microscope, two groups of investigators showed that the endothelium does not regenerate by cellular division following injury but that the remaining cells spread and enlarge in order to cover the space left by loss of injured cells.[39,40] According to

Van Horn, the decrease in endothelial cell density following cataract extraction could make the patient susceptible to further insult.[41] In the rabbit cornea, the situation is different. Endothelium of the rabbit cornea regenerated following injury by cell division of cells at the margin of the wound. New cells migrated and replaced destroyed cells. Van Horn found that the regenerative capacity of the corneal endothelium of the cat was similar to that of man and other primates.[41] The cat appears to be a good, inexpensive model to study ultrastructure and function of the stressed corneal endothelium.

Other interesting investigations concerned changes in the corneal endothelium as a function of age.[40] These changes were measured following in vivo specular photomicrography. Sixty-one normal volunteers of both sexes ranging in age from 20 to 89 years were studied. Endothelial photographs were taken of the cellular area located in the central cornea. Cells were measured and the mean endothelial cell area was found to double approximately from age 20 through 80. When these investigators, using the clinical specular microscope, photographed the endothelium of eyes of fifteen patients who had undergone successful penetrating keratoplasty, they found that the average endothelial cell area was one to six times larger and that the average cell perimeter was two and a half times larger than that of a normal cornea of a subject of the same age as the donor.[42] The thickness and transparency of each graft was normal. In general, the aging process and trauma apparently had similar effects, namely a loss of destroyed cells with an increase in the size of remaining cells in order to cover a given denuded area.

By means of transmission electron microscopy, the addition of reduced glutathione (GSH) to a bicarbonate Ringer's solution, during in vitro perfusion of the corneal endothelium was shown to maintain endothelial ultrastructure, to increase the efficiency of the endothelial pump, and to prevent depletion of cellular ATP.[43] Diamide, a thiol-oxidizing agent, which stoichiometrically oxidizes intracellular glutathione to the disulfide (GSSG) has been used to study the temporary effects of GSH. The results of Edelhauser and colleagues suggest that the ratio of reduced to oxidized glutathione in endothelial cells play a role in the maintaining endothelial cell barrier function.[43] This observation is a step toward trying to define the physiological and biochemical roles of glutathione in living cells.

In addition to the above, it was found that endothelial cell damage to isolated rabbit and human corneas perfused in vitro resulted in increased corneal thickness due to incomplete composition of 0.9 percent NaCl, Plasma-lyte 148, and lactated Ringer's solution.[44] Endothelial cell damage could be prevented if the intraocular irrigating solution contained concentrations of inorganic and organic constituents that were similar to those found in aqueous humor.

The contribution of electron microscopy to the study of corneal pathology should be documented.[45] Keratoplasty was shown to be the most important source of specimens for ultramicroscopic investigation. By means of these techniques it has been possible to confirm many light microscopic studies in pathological corneas. Such investigations have been helpful in identifying sites and types of pathological changes in corneal dystrophies and degenerations. Also, it has been possible to observe histologic effects of corneal inflammatory conditions as well as of metabolic disorders affecting corneal transparency.

Scanning electron microscopy has proved to be a very useful tool. This was brought to the attention of those who participated in a Workshop on Biomedical Applications of Scanning Electron Microscopy (SEM) held in 1976.[46] On this occasion, Van Horn presented and published subsequently a report which concerned corneal endothelial regeneration, a comparative study in the rabbit and cat using scanning electron microscopy techniques.[46]

A very progressive look, presented by Hart, concerned biosynthesis of glycosamino-glycans during corneal development.[47] This investigator was interested in the role of keratan sulfate in the regulation of corneal transparency. He observed the rate of incorporation of ^{3}H and ^{35}S-labeled keratan sulfates into glycosaminoglycans up to and beyond the fourteenth day. The proportion of ^{3}H and ^{35}S in keratan sulfates reached near maximum levels as early as the ninth day. On and after the fourteenth day, keratan sulfate appeared to become more highly sulfated. Hart suggested that these and other changing patterns of glycosaminoglycan biosyntheses during corneal development play an important role in corneal morphogenesis and in the development of corneal transparency.

Numerous studies regarding corneal hydration, transport and storage have provided important information.[41, 48-54] Bowman and Green used the specular microscope to determine the effect of varying hydrostatic pressure on the thinning rate of pre-swollen de-epithelialized or de-endothelialized cornea.[48] De-epithelialized corneas thinned more slowly as hydrostatic pressure on the posterior surface was increased, until fluid movement stopped at 60 to 70mm Hg pressure. Fluid moved from the stroma toward the aqueous humor against considerable hydrostatic pressure. De-endothelialized corneas thinned at a higher rate as hydrostatic pressure was increased forcing fluid out across the epithelium, indicating that this effect was probably mechanical with increasing pressure. Therefore, the fluid was shown to move against an increased hydrostatic pressure. These investigators concluded that there must have been a considerable physiologic force which caused movement across the normal endothelium.

Studies to understand endothelial physiology were proposed by Van Horn and associates.[41] Investigations by Maurice and colleagues failed to show any differences between the pumping and non-pumping state of the cornea in the behavior of inorganic ions (^{22}Na, ^{36}Cl, $H^{14}CO_3$).[49] They concluded that they were dealing with either a neutral salt pump, a bulk flow pump, or a bicarbonate pump. For other solutes the permeability of the endothelium appeared to bear a linear relationship to the free diffusion constant of the molecule. Their observations continue to suggest that solutes pass the cell layer by paracellular pathways. Development of an automatic volumetric technique designed to measure fluid movement across the endothelial layer allowed investigators to achieve resolution down to one nanoliter.[50,51] Using this refined technique, the presence of 5mm adenosine was found to stimulate fluid transport by as much as 50% when the rates measured were compared to controls in which adenosine was omitted. Data from calculations by Fischbarg and Lim suggest that fluid flows partially through a route other than the cells' membrane proper.[50,51] Though additional experiments are necessary, they suggest that the standing-gradient model might not be appropriate for the endothelial layer.

Insulin was found to have a biphasic effect on fluid transport, stimulating it at concentrations lower than 10^{-10}M but inhibiting it at concentrations larger than 10^{-8}M. Ouabain was found to stimulate transport below 10^{-9}M and inhibit it above 10^{-7}M. These findings raised the question that perhaps the insulin effect might be explained in terms of its modulation of transport ATPase activity. At present, this group is considering more theoretical aspects of the fluid pumping mechanisms.[50,51]

Immune Response, Corneal Transplantation, and Storage

Two interesting experimental systems were developed by Maurice and Perlman which may be useful to others in studies involving immune response.[55] They devised an experimental method of destroying rabbit endothelium by the use of benzalkonium chloride.[49] Then they plated endothelial cell suspensions onto denuded rabbit stroma and used this tissue to graft onto de-endothelialized eyes. Preliminary studies showed that 5 x 10^6 cells per cornea would produce a uniform monolayer. Experimental transplant studies in rabbit eyes are in progress.

Maurice and co-workers developed a leukopenic rabbit model by administering two whole-body irradiation exposures (1400 roentgens per dose at a two-day interval, with eyes shielded).[49] By the fourth day following this treatment, the peripheral white blood cell count was 0.5 percent that of normal values. Experiments using normal and irradiated rabbits were carried out. In eyes injured by means of thermal cautery made 2mm from the limbus, vessel growth occurred by the third to fourth day. The technique demonstrated that vascularization did occur in leukopenic animals. Animals treated as described might be useful in many types of related experiments which have to do with the immune response.

This year, studies in ocular immunology dealt with many facets of immunology. Allansmith remarked that according to current methods, neither HL-A nor ABO typing predicted the outcome of corneal transplantation.[56] Neither ABO nor HL-A incompatibility could be shown to be factors in corneal graft failures.

Immunoglobulin assays were made in several systems. Tissue, tear and serum IgE concentrations were measured in normal subjects and compared with preparations taken from patients with vernal conjunctivitis.[57] Allansmith and co-workers questioned whether an immunogenesis of IgE plasma cells occurred in areas of the upper conjunctiva in vernal conjunctivities.[57] Half of these patients had abundant IgA, IgD, and IgE forming plasma cells. These immunoglobulin forming plasma cells were absent in normal subjects. Tear IgE values did not vary significantly in normal and patient assays. Serum IgE was much higher for patients than for normal subjects. Allansmith, et al concluded that their findings were consistent with hyperplasia of IgA, IgD, and IgE antibody-forming cells in the tarsal conjunctiva of some patients with vernal conjunctivitis. [57]

In other experiments estimates of the numbers of plasma cells in the main lacrimal gland ($3.2x10^6$), the conjunctiva ($2.1x10^6$) and in the accessory glands ($1.8x10^5$) were made.[58] Since there were so many cells in the relatively

inaccessible lacrimal gland, it was suggested that cells of the lymphatic series were attracted to the lacrimal gland area by non-antigenic mechanisms.

Abelson and associates demonstrated histamine in human tears, which indicated a possible role for this mediator in both physiologic and immunologic processes of the external eye.[59]

Khodadoust and Silverstein described studies that extended their earlier work on lymphocyte-mediated destruction of corneal grafts.[60] In the rabbit, destruction of the histo-incompatible corneal endothelium was marked by the formation of focal pock-like damaged areas, rather than by the typical moving line of rejecting endothelium usually seen in spontaneous graft rejection. Where the transferred lymphoid cells were compatible with the tissue of the graft recipient, the picture was one of a severely affected graft on a field of uninvolved recipient corneal endothelium. Where the lymphoid cells were compatible with the graft and not with the tissues of the recipient, a clear corneal graft survived on a bed of endothelial destruction. These studies have contributed an experimental model for immunological testing of graft-versus-host reactions.

Syndromes associated with conjunctivitis and other immunological ocular complications of contact lens wearers were studied.[61,62] Allansmith reported that microorganisms served as antigens to initiate an immune response in human subjects.[61] Bacteria present on lid margins were found to shed products into tear films. Normally these products were washed away but not in cases where people wore contact lenses. Particles stuck to lens surfaces and acted as stimuli to produce hypersentivitiy reactions.

Another distressing syndrome observed in both hard and soft contact lens wearers was characterized by increased mucus, itching, decreased lens tolerance and giant papillae formation in the upper tarsal conjunctiva.[62] Biopsies of the upper tarsal conjunctiva revealed basophils, eosinophils, mast cells, lymphocytes, and plasma cells. Allansmith, et al postulated that the syndrome was immunological in origin and that it was a major cause of difficulty in wearing contact lenses.[62]

Obviously, immune response in the cornea must be documented further and characterized in order to provide measures for prevention and treatment of the various complications. As a consequence, in order to stimulate increased research interest and activity in the field of ocular immunology, a grant announcement entitled "Immunological Effects on Corneal Disease Processes", is in preparation. Also, an international symposium planned by Dr. A. M. Silverstein, "Ocular Immunology and Immunopathology", will be held in May 1978.

The consensus of the scientific community is that studies concerning ocular immunology, especially those pertaining to the cornea carry a high priority value at this time. To address ongoing investigations with continued fervor and to expand the program where limitations are apparent is an important aim of the present National Eye Institute Corneal Disease program.

Finally, in relation to storage, problems which encompass various immune phenomena deserve the attention of clinical ophthalmologists.

Continued studies by Kaufman and associates with M-K medium, used for storage of corneal tissue at refrigerator temperature, showed that 80 percent of 92 human corneas stored for up to 7 days in this simple tissue culture medium remained clear and thin 2 months after keratoplasty.[63] Deturgescence rates of tissue stored in this medium compared favorably with those for fresh and cryopreserved tissue. Hull and co-workers showed that dextran passed into rabbit corneas stored in M-K medium prior to corneal transplantation.[64-66] In about 24 hours, an equilibrium between dextran in the cornea and M-K medium was reached. Post-keratoplasty dextran efflux was rapid with about 70 percent to 75 percent of the compound lost in 12 hours.

During a three year interval, Doughman and colleagues maintained numerous corneal organ cultures at 37° in order to study changes with time and to establish a model for experimental pathology.[67] Many animal studies were conducted before experiments with human tissues were performed. Human corneas retained in organ culture maintained ultrastructural integrity, glucose metabolism, normal levels of lysosomal and cytoplasmic enzymes and physiological deturgescence for at least 35 days. Clinically, 75 penetrating keratoplasties using corneas incubated for an average of 14 days were performed with results as good as those obtained when currently accepted storage methods were used. There were no problems with infected tissues. A significant finding in animal model systems was that with chicken to rabbit transplant experiments, xenograft rejection was delayed or prevented when corneas had been maintained three weeks in organ culture. Numerous assessment studies related to the above findings were reported to be underway.

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CATARACT

Introduction

Cataract, an opacification in the lens of the eye, interferes with the passage of light through the eye and results in visual impairment. This disorder accounts for approximately one-sixth of all cases of visual impairment in the United States. There are about 300,000 operations to remove cataracts performed each year, yet there are an additional 1,670,000 Americans who have difficulty seeing because of cataracts developing in one or both eyes. Cataracts have necessitated approximately 2,725,000 annual visits to eye doctors in recent years. The physical limitations imposed on the afflicted individuals and the cost to the public as a result of cataracts are considerable.[1]

The varieties of cataract affecting humans and encompassed by the National Eye Institute's Cataract program include senile cataract; diabetic cataract; congenital, metabolic and genetic cataracts; and cataracts induced by drugs and radiation, and those which occur secondary to other eye disorders. The Cataract program is also concerned with such subjects as accommodation and optical problems related to cataract and aphakia.

The research interests of the Cataract program include determination of the causes of cataract, means of preventing or retarding cataract development, prevention of amblyopia in children with cataract, development of new methods for correcting optical problems following cataract surgery, evaluation of the safety and efficacy of new methods of cataract extraction, and efforts to improve the life cycle and adaptation to new spatial relationships of patients who have undergone cataract surgery.

Interdisciplinary approaches to studies of the normal and cataractous lens are currently gaining favor in the quest for knowledge related to cataractogenesis. These studies employ the evolving methodologies and technologies of such diverse disciplines as anatomy, embryology, cellular and molecular biology, genetics, biochemistry, physical chemistry, physiology, optics, microbiology, immunology, pharmacology, pathology, experimental ophthalmology, epidemiology, bioengineering and clinical research (with controlled clinical trials rapidly becoming an important aspect of the latter).

Several hypotheses have helped shape the nature of contemporary research on the lens and on the physiochemical mechanisms underlying lens transparency. Current research emphasizes the importance of controlled hydration,[2] as well as the size and state of lens protein molecules[3] in determining the ability of the lens to transmit and refract light properly. Hypotheses being tested include the concept of osmotic shock as a factor in the alteration of cell relationships and subsequent development of lens opacification, the thesis of protein change with proteolysis or aggregation of proteins as factors in the disruption of light transmission, and the possibility that transport mechanism abnormalities, with consequent alteration of metabolism, may contribute to cataract formation.

Previous Annual Reports of the National Eye Institute, particularly those from FY 1973 through FY 1976, provide examples of the avenues of research being

pursued in many of the subprograms listed above. The present report emphasizes some of the recent initiatives undertaken by the vision research community in an effort to exploit and refine the scientific and technological advances of prior years.

Cooperative Cataract Research Group

A number of investigators in cataract research have organized themselves into a Cooperative Cataract Research Group (CCRG). The concept of the group was unveiled at the meeting of the Association for Research in Vision and Ophthalmology in early 1976 and received the endorsement of the National Advisory Eye Council in late 1976. Those forming the cooperative group believed that despite the significant advances achieved in research on animal cataracts, only limited progress had been made toward understanding the nature of human cataracts. Thus, a concerted effort on human cataracts is needed. The CCRG's members believe that the problem can best be approached by a well-coordinated team of experts representing a variety of research disciplines. The intention of the CCRG is to foster closer collaboration among investigators who are widely dispersed geographically.

The scope of intent, operations, and needs of the CCRG is captured within the Proposed Research Program for the Cooperative Cataract Research Group.*

> "The immediate objective of the proposed collaborative effort is to establish various morphological, biochemical, physiological and biophysical characteristics for the human lens since there is little reliable data available at the present time. The second step is to assess the changes in these parameters as the result of the cataractous process. A successful program in human cataract research is feasible by combining the technology that is already available in several laboratories in a coordinated fashion. This approach also has the advantage that with only slight additional effort in each of the laboratories where already active research is going on, the proposed studies can be successfully carried out without the need of developing or refining new techniques."

The CCRG has arranged for the collection and distribution of normal and cataractous human lenses throughout the United States and abroad. These lenses are to be obtained from eye banks, at autopsy, and as a result of surgical procedures involving cataract removal or enucleation. The CCRG has adopted standard practices for handling, photographic recording, classifying, storing and shipping cataractous and normal human lenses. The cooperative group's research data will be collected and disseminated from a coordinating center located in the Laboratory of Vision Research, National Eye Institute. Telephone conferences and meetings of cooperative group participants to examine

*"Characterization of Human Lens in Health and Disease", Minutes of Meeting, NAEC, Sept. 13-14, 1976 p. 11-13.

results and plan future experiments are to be held periodically. Members of the CCRG's Executive Committee are G. W. Barber, Wills Eye Hospital, Philadelphia, Pennsylvania; L. T. Chylack, Harvard Medical School, Boston, Massachusetts; E. Cotlier, Yale University, New Haven, Connecticut; J. E. Harris, University of Minnesota, Minneapolis, Minnesota; V. E. Kinsey and V. N. Reddy, Oakland University, Rochester, Michigan; A. Spector, Columbia University, New York, New York; and J. H. Kinoshita, National Eye Institute, Bethesda, Maryland.

To fund the CCRG's initial activities, the National Eye Institute has provided one-time administrative supplements to the pre-existing grants of eighteen extramural CCGR participants.

The CCRG may well serve as a model for close collaboration by a number of geographically dispersed NEI grantees who are interested in sharing materials and data related to a specific health problem. The coming years will certainly test the value of this collaborative mechanism.

Irradiation-Induced Alterations in Lens Cells

X-irradiation of the lens continues to interest investigators who believe that the rapidly elicited cellular dysfunctions produced in this model may be comparable to events occurring after other more insidious forms of cataractogenic insults. However, utilization of the model depends, in part, upon defining the locale and nature of those cells susceptible to X-irradiation. Worgul and colleagues, using autoradiographic techniques, i.e. incorporation of tritiated thymidine into dividing cells, have provided evidence that the cells of the germinative zone of the lens epithelium behave in a demonstrably different fashion following X-irradiation.[4] Cells derived from the germinative zone at the time of irradiation gradually develop atypical nuclear characteristics and undertake an unusual migration to the posterior region of the lens where cortical cataract is observed shortly thereafter. Identification of the critical cell population affected by X-irridation permits greater accuracy in future studies directed at determining the intracellular malfunctions reponsible for the formation of cataract.

Role of Glutathione in Maintaining Lens Transparency

Glutathione has been studied extensively in the lens, yet considerable controversy remains regarding its actual physiological role and its possible relationship to maintaining lens transparency. Glutathione concentration in the lens is normally very high but decreases steadily with age and in the course of the formation of practically all cataracts. However, whether the decrease of glutathione initiates or is only coincidental with cataractogenesis is not known. In recent studies Giblin and coworkers have investigated the relationship of glutathione to the transport function of the lens epithelium.[5] Their findings indicate that the concentration of glutathione in the epithelial cells is five-fold greater than in other parts of the lens and that the oxidation of glutathione leads to pronounced changes in this tissue. The changes include: (1) reduction in the activity of Na^+-K^+ATPase; (2) shift in the distribution of sodium, potassium, and chloride ions; (3)

increase in hydration; and (4) decrease in the active transport as well as increase in the passive diffusion of labelled-rubidium. The inactivation of Na+-K+ATPase, resulting from the depletion of glutathione, is of considerable interest since several types of cataracts, including those of the Nakano strain of mice, are characterized by a 50% reduction in the Na+-K+ATPase of those lenses which ultimately become cataractous. Reversing the consequences of glutathione depletion remains an elusive yet challenging aspect of research directed at understanding the development of cataracts as well as a medical treatment for this disorder.

Aggregation of Lens Proteins and Cataractogenesis

Earlier studies of the lens have revealed the age-dependent increase in the amount of the water-insoluble albuminoid fraction and soluble high molecular weight aggregates. Lens protein aggregates of greater than 50 million daltons from several mammalian lenses, including that of man, have been isolated and characterized. Electron microscopy has also demonstrated the presence of large protein clusters in the cataractous human lens. These aggregates are large enough to scatter light in the visible range, and at sufficiently high concentration they may lead to lenticular opacification. An understanding of the formation of senile cataract therefore requires knowledge of the mechanism of aggregation and insolubilization of lens proteins.

The aggregation of a well-defined lens protein, bovine alpha-crystallin, has been under increasing scrutiny because: (1) it is the best characterized macromolecular lens protein and appears to be the only soluble macromolecular crystallin present in medium of high density and viscosity, simulating the in vivo condition, and (2) the low molecular weight form found in young fibers, containing more than 40 polypeptide chains, is the primary contributor to the formation of high molecular weight and insoluble proteins in the bovine lens.

Li and collaborators have conducted studies to delineate the aggregation and interaction of the subunits of calf low molecular weight alpha-crystallin.[6] Carboxymethylation of the A chain polypeptide with iodoacetic acid resulted in a drastic decrease to half the original size of this protein. A maximum of 80% of the total sulfhydryl groups of the native protein can be altered, indicating that the remaining 20% are blocked or situated in the interior of the alpha-crystallin macromolecule. No change in the polypeptide backbone conformation was detected. Histidine and tyrosine residues were not involved in the decrease in size of the carboxymethylated native protein. The native macromolecule is visualized as a dimer of 12 S components that is stabilized cooperatively by a cysteine site and a hydrophobic site. This is the first demonstration of the formation of a discrete entity by the modification of no more than one sulfhydryl group per A chain polypeptide.

Conversely, iodination of the tyrosine residues of low molecular weight alpha-crystallin leads to gradual increases in the size of the protein. A maximum aggregate size 28S was obtained when all the tyrosines were modified.

The foregoing results demonstrate that variously sized populations of low molecular weight alpha-crystallin can be prepared through chemical modification.

Knowledge of the formation of these differently sized aggregates provides an understanding of the role of functional groups in causing increased aggregation of lens proteins. Researchers believe that once the dynamics of the aggregation of the macromolecule are understood similar analysis may be applied in studies of human tissues. Insights thus acquired may ultimately lead to a reasoned approach to prevention, delay, and reversal of cataracts.

Medical Approaches to Cataract Treatment and Prevention

Since the events leading to the formation of senile cataract remain relatively obscure, there is as yet little firm scientific basis from which to propose a rational medical treatment of the disorder. A particularly distressing hindrance is the absence of an animal model that is truly representative of human senile cataract.

Sugar cataracts, including the diabetic variety, appear to offer a more substantial footing upon which to base treatment aimed at preventing, delaying or reversing lens opacifications due to metabolic dysfunctions associated with carbohydrate metabolism. Aldose reductase catalyzes the conversion of glucose and galactose to their respective alcohols. Evidently these polyols accummulate within the lens resulting in osmotic swelling, loss of selective permeability and, ultimately, lens opacification. Thus inhibitors of aldose reductase have been tested in an effort to prevent or delay the cataractous process, and some success has been achieved in delaying the onset of cataracts in galactosemic animals.

The clinical implications of such findings are as yet unclear since lens problems associated with galactosemia can be dealt with rather easily by withdrawing galactose from the diet.[2] In diabetes the problem is somewhat more complex. Though the hyperglycemia of diabetes can be controlled by insulin therapy and/or diet, many complications associated with the disorder appear to go unchecked. Furthermore, synergistic effects, i.e. environmental and genetic factors plus diabetes, have been suggested as contributing to the earlier onset and maturation of cataracts in diabetics. If this proves to be the case then long term therapy, i.e. the administration of appropriate aldose reductase inhibitors, could be of appreciable benefit to diabetics.[2, 7]

A subject that has been receiving increasing attention recently is the function of the enzyme superoxide dismutase, an inactivator of the potentially harmful superoxide radical. The superoxide radical is generated in various enzymatic and non-enzymatic oxidation-reduction reactions, with light often the catalyst. Since the lens contains compounds with the capacity to produce the superoxide radical and is also the recipient of direct photochemical energy there is a need to understand how the lens protects itself from the insults rendered by the radical. Studies have shown that superoxide dismutase is present in the lens,[8] though primarily in the lens epithelium.[9, 10] Thus lens fibers lack superoxide dismutase, as well as other enzymes, e.g. catalase, that protect against the superoxide radical and its derivatives. Kuck has suggested that "this lack of protection may be critical in the human lens nucleus where the normal pigment absorbs long-wave ultraviolet light, trapping energy which can decompose sensitive molecules or produce polymerization."[10]

The latter may be especially relevant in light of Spector's studies suggesting that gradual reorganization of proteins within the lens nucleus is responsible for loss of lens transparency.[3]

Diabetic Cataract

As stated above, sugar cataracts are provoked by radical changes in lens hydration resulting from the accumulation of large amounts of sugar alcohols. The conversion of cataractogenic sugars to these polyols is catalyzed by the enzyme aldose reductase. Nevertheless, these cataracts can be retarded or prevented by lowering the polyol levels in the lens either by withdrawing the offending sugar or with inhibitors of aldose reductase.

In the case of diabetes a number of aspects of the inappropriate metabolism of glucose are currently under scrutiny. Coulter and Knebel are endeavoring to determine whether insulin directly influences lens metabolism under physiologic conditions.[11] They have developed a sensitive radioimmune assay to measure insulin in both aqueous humor and blood plasma. Observing fasted and fed animals, they have shown that the concentration of insulin in aqueous humor is correlated with the concentration of the hormone in plasma if allowance is made for the relatively slow rate of aqueous humor formation. However, the concentration of insulin in aqueous humor is only 2% that of plasma, suggesting that the blood-aqueous barrier is relatively impermeable to insulin under normal conditions. Changes in the availability of insulin to the lens, i.e. directly from the aqueous humor, could conceivably lead to disordered lens metabolism in diabetes. Sheaff and Doughty have been studying the chemical, physical and biological properties of the enzyme aldose reductace, with the expectation that a fuller understanding of this catalyst may permit more effective inhibition or manipulation of the enzyme in the living organism.[12]

Lens Implants

Although surgery for the removal of cataracts is highly refined and successful, subsequent visual correction may not always be fully satisfactory. Correction with spectacles, although acceptable to many, may be unacceptable to others as a result of differences in image size, lens abberations, reduction in the visual field, wearing discomfort and, in the case of unilateral aphakia, difficulty in fusing the images of the two eyes. Correction with contact lenses may resolve some of the aforementioned problems, but a number of difficulties may remain including requisite patient dexterity.

Even then, some individuals with unilateral aphakia lack the ability to fuse images. As a result of the limitations associated with spectacles and contact lenses, the intraocular lens, i.e. a plastic prosthesis surgically implanted into the eye, is increasingly being used as an alternative means for the correction of sight following cataract removal. The advantage of intraocular lenses include placement of the corrective lens in a near normal position within the eye permitting improved visual correction, and the diminished attention to the prosthesis required of the patient.

With improvements in the design of lenses for intraocular implantation, the number of such implants has been increasing at an exponential rate. Many

feel that it is important at this time to evaluate carefully the efficacy of such implants in patients undergoing cataract extraction and to assess their risks as well.

As a first step toward evaluating intraocular lens implants, the National Eye Institute will attempt to summarize the experience that ophthalmologists have had in their recent clinical practice. The records of several surgeons will be analyzed to see whether they can provide answers to research questions that are of interest. For findings from the study of such records to be credible, it will be necessary to demonstrate that data from all patients treated are included, that follow-up was maintained on a large proportion of cases, and that significant medical data have been carefully recorded.

Demonstrating that these elements are present constitutes the work scope of a contract which is to be awarded in the fall of 1977. If in the first year of the contract the feasibility of a high quality retrospective study is established, the contract will be extended to analyze and report on the benefits and risks of intraocular lens implants. Consideration is also being given to more comprehensive research designs, such as prospective controlled studies, perhaps including randomization, but it is not entirely clear at this time that these are feasible.

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GLAUCOMA

Introduction

With the exception of the type termed low-tension, glaucoma constitutes a group of debilitating diseases which are characterized by increased intraocular pressure (IOP) with subsequent alterations in the optic nerve and eventual loss of visual field. The Glaucoma program of the National Eye Institute supports clinical and laboratory investigations of factors regulating intraocular pressure and disorders thereof, study of related changes in the optic nervehead, and appraisal of the visual field. The preponderance of research supported by the Glaucoma program has been physiologic and pharmacologic studies directed at understanding the mechanisms of aqueous humor formation and aqueous egress as well as at their control. Glaucoma research at present reflects an uneven distribution of effort because the disease is primarily observed in humans. Available animal models such as congenital glaucoma in rabbits and beagles and ocular hypertension induced in monkeys by use of prostaglandins are of limited usefulness.

Glaucoma is a common and serious eye disease. Based upon statistics of first visits to physicians (exclusive of referrals) there appear to be approximately 178,000 new cases of glaucoma each year. There is a considerably higher incidence of the disease in females, and new cases are first diagnosed predominately in individuals at ages 45 years or older. The estimated prevalence of impaired vision resulting from glaucoma is approximately 1,070,000. Severely impaired vision is observed in 207,000 of these people, and about 56,000 of these, mainly at ages 65 or over, are considered legally blind as a result of glaucoma.

Eye surgery for glaucoma represents about 3% of all ophthalmic surgery, and surgical costs for this disease are estimated to be $8,758,000 per year. A large majority of glaucomatous patients are treated by means of eyedrops and other medications rather than by surgery. In the United States, the cost for one year for one type of eyedrop alone used for treatment was $29,000,000. Thus, besides being a widespread and serious eye disease, glaucoma, is also a costly one.

The National Eye Institute supports research dealing not only with the etiology of glaucoma but also with improving its diagnosis and developing new modalities for its treatment. Methods are currently being developed to achieve greater accuracy in the measurement of intraocular pressure, rate of aqueous humor production, facility of outflow, and extent of damage to the optic disc and associated vasculature and nerves. In addition, development of improved means of pharmacological and surgical treatment of the various types of glaucoma is in progress.

Medical Treatment of Glaucoma

Medical treatment of primary glaucoma continues to rely upon the use of an established arsenal of drugs. Such drugs are of the parasympathomimetic or cholinergic type, such as pilocarpine and carbachol which are short-acting,

and Phospholine Iodide and demecarium bromide which are long-acting. Another group of compounds used topically are the sympathomimetics or adrenergics which constitute various preparations of epinephrine. Oral drugs act systemically and reduce aqueous formation by inhibition of carbonic anhydrase in the ciliary processes or by short-lived lowering of the IOP through hyperosmolarity. Examples of the former type are acetazolamide and methazolamide; glycerol is representative of the latter group.

Drug Delivery Systems. Parasympathomimetic preparations like pilocarpine cause miosis and may produce side effects due to spasm of the ciliary muscle, resulting in myopia and blurred vision. To obviate such deleterious effects and enhance efficient utilization, newer methods of administering drugs are under investigation. For short term hypotensive effects, soft contact lenses have been soaked in pilocarpine for several minutes and then worn for time periods up to one day.[1]

A more recently developed device which can provide a rate-controlled delivery of the drug for up to a week is the Ocusert developed by the Alza corporation. The delivery system consists of two hydrophobic copolymer membranes between which pilocarpine is sandwiched as the free base. The Ocusert is placed under the lower lid. When the polymer comes in contact with the tear film, the drug diffuses through the polymer membranes at a predetermined rate. Although occasionally a "burst phenomenon" occurs causing an over-release of pilocarpine, the Ocusert's primary disadvantage has been its high cost. The Ocusert, however, appears to be a safe device offering several advantages over eyedrops. Favorable aspects of its clinical usefulness continue to be reported.[2]

A new topical drug has been developed which holds great promise for the treatment of glaucoma if its long-term use does not reveal a loss in effectiveness. Timolol is a beta adrenergic blocker which has no known side effects and can control IOP for 24 hours by use of a single eyedrop. The drug does not appear to act by increasing the facility of aqueous outflow.[3] An NEI grant-supported clinical trial of timolol for both basic pharmacological and clinical evaluation is now in progress.

Genetic Aspects of the Etiology of Open-Angle Glaucoma

The widely held concept that certain individuals have a genetic predisposition to glaucoma has recently been strengthened by the observation by Shin and associates that there is an increased prevalence of certain histocompatibility antigens, namely the HLA-B7 and HLA-B12 antigens on lymphocytes, in patients suffering with primary open-angle glaucoma as compared to the general population or patients with normal IOP.[4] Furthermore, the presence of either antigen also appears to be an important prognostic indicator of the development of visual field loss in patients with increased IOP and in those who are high responders to topical corticosteroids.[5]

Responders were classified on the basis of their IOP response to the topical steroid dexamethasone: NN-NG, initial IOP less than 21 mm Hg increasing to less than 32 mm HG after the testing period, and GG, initial IOP more than 20 mm HG increasing to more than 31 mm HG after the testing period.

Shin and associates then extended their studies to subjects who did not have glaucoma.[6] The presence of either HLA-B7 or HLA-B12 antigens was found to be associated with a higher prevalence of cup/optic disc (C/D) ratios of more than 0.3 in GG responders and in the combined NN-NG groups. The C/D ratio was previously determined to be inherited and larger C/D ratios in individuals were thought by Armaly to be related to increased IOP,[7] by Becker to increased responsiveness to topical corticosteroids,[8] and perhaps to an increased sensitivity to glaucomatous damage. Presence of either HLA antigen was found by Shin and associates also to be associated with a higher prevalence of a family history of glaucoma in the GG group. This suggests that either of the histocompatibility genes themselves or an additional gene on chromosome six may be associated with the inheritance of expression of primary open-angle glaucoma. No associations were noted in non-glaucomatous individuals between these antigens and their age, race or mean IOP in either of the groups studied.

Inflammatory Reactions Associated with Secondary Glaucoma

Etiology. Glaucoma secondary to inflammation of the uveal tract occurs in many different forms and is considered one of the most damaging types of glaucoma seen. Despite numerous published clinical reports about this condition, little is known about the etiology of uveitis.[9] The lack of sufficient research in this area has been taken into account in the preparation of a recent NEI grant announcement which invites the submission of worthy grant proposals focused on clinical or laboratory research that deal with secondary glaucomas.

Most of the ongoing research on glaucoma secondary to uveitis is concentrated on animal experiments in which prostaglandins and related substances are used to produce inflammatory reactions resembling acute uveitis that cause sharp rise in IOP.[10] These experiments have raised speculation that prostaglandins and related compounds may have a role in human uveitis and associated secondary glaucoma[11] even though there are important species differences in response to prostaglandins.[12] Further research is needed to determine whether this class of compounds does in fact have a true etiologic role in this condition.

More study is also needed to explain why IOP is elevated in some cases of uveitis and not in others, and what actually occurs in the aqueous outflow channels or in the ciliary body to cause this condition. Clinicopathologic study of aqueous humor and anterior segment tissue samples removed during surgery are promising approaches. In addition, there should be more study of uveitis which is experimentally induced in primates in order to understand better the immunological mechanisms involved in the inflammatory response.

Approaches to Treatment. Effective use of anti-inflammatory corticosteroid drugs in the eye has significantly improved the treatment of various forms of uveitis and associated secondary glaucomas. Steroids have also been found valuable in reducing tissue reactions to antiglaucoma surgery, thereby raising the success rate of this procedure. Nevertheless, safer and more effective therapeutic agents are needed for treating this condition because of possible side effects from steroid therapy such as cataract formation.

Axoplasmic Flow in Nerve Fibers in the Etiology of Glaucoma

"Axoplasmic transport" is the term applied to the movement of proteins, sugars and subcellular particles from the nerve cell perikaryon along its axon. The functional role of this transport is not yet completely understood, but appears associated with nerve cell function and maintenance.[13] Axoplasmic movement consists of several components: a slow component which moves at a rate of 1 to 2mm/24 hr and consists mainly of microtubules and neurofilaments, and a fast component which carries phospholipids, enzymes, neurotransmitters and endoplasmic reticulum and moves at a rate of 400mm/24 hr.

Besides an orthograde flow from retinal ganglion cells where the axoplasmic flow is synthesized to the lateral geniculate nucleus, there is a retrograde flow from the lateral geniculate nucleus to the retina at a rate of 75 to 150mm/24 hr. The slow moving component is blocked at the optic disc by mechanical ligation, but recovers when such ligation is removed. The fast moving component is known to be oxygen-dependent and is irreversibly blocked after six hours of ischemia in vivo. The fast moving transport depends on the structural integrity of microtubules carried by the slow transport. If the structure of the microtubules is disturbed, the fast transport and transsynaptic conduction cease.

In experimental work on owl monkeys,[14] Anderson and Hendrickson reported that, with a moderate rise in IOP, labelled protein synthesized in the retinal ganglion cells was observed to be partially obstructed at the lamina cribosa; a further rise of pressure to within 25 mm HG of the mean blood pressure caused complete cessation of fast transport. Retinal synthesis of transport material ceased when the IOP exceeded blood pressure.

Such studies were extended by Minckler and associates to another species of monkey, in which orthograde and retrograde axoplasmic transport were separated by use of tritiated retinal ganglion proteins and horseradish peroxidase (HPR) injected into lateral geniculate nuclei.[15] Both tracers accumulated at the lamina cribosa of eyes maintained at elevated IOPs for 12 to 28 hours, but HPR appeared to be a much more sensitive indicator. The degree of retrograde transport obstruction appeared to be directly proportional to both the degree and duration of the elevated IOP. Through the use of serial reconstructions of radioautographs and peroxidase-reacted sections of the optic nerve heads, it was demonstrated by light microscopy that these obstructions were not only in the lamina cribosa but also occurred in the temporal quadrants of the nerve head. At elevated IOPs, these transport obstructions occurred despite evidence of elevated arterial P_{O_2} levels or intact nerve head capillary circulation.

These studies support suggestions that alterations in axoplasmic transport may play a role in the pathogenesis of visual damage in human glaucoma. Transport obstructions were demonstrated in the lamina cribosa in acute experiments in monkeys at IOPs comparable to those characteristic of human chronic open-angle glaucoma. The occurrence of such transport blocks were most pronounced in the temporal quadrants of the nerve head, the same quadrants which are most often affected in the human disease. Compromise of the axoplasmic transport

could play a role in the continuous destruction of axons, causing gradual loss of visual field, that is typically observed in glaucoma. This damage appears to a certain extent to be reversible as does visual damage in the early stages of the disease. Since the precise function of retrograde transport in axons as well as the role of optic nervehead circulation in this process is unknown, much additional work must be done before the pathogenesis of visual damage in glaucoma is understood.

In normal eyes rhythmic fluctuations in the IOP occur during a 24 hr cycle, but a pressure change of more than 2 or 3mm HG seldom occurs. However, in many glaucomatous eyes, the pressure varies in a definite diurnal pattern which is an exaggeration of the slight normal pressure variations. A peculiarity of the diurnal variation of IOP in glaucoma is that in most patients eye pressure rises to a maximum early in the morning before they rise while in others the pressure reaches its maximum either in late afternoon or evening. Weitzman and associates have reported significant 24 hr temporal relationship between IOP and the episodic pattern of cortisol secretion in normal subjects and in patients with different types of glaucoma.[16] A phase difference of 3 hrs has been observed between the maximal values of these two measures.

The need for more investigations to determine relationships between various hormonal and neural influences which impart a circadian rhythm to IOP in normal individuals as well as in glaucoma patients has prompted the idea of conducting a NEI workshop on this topic in early 1978. Since biological rhythms have different effects on several ocular tissues, the scope of this subject could be expanded to encompass the work of scientists in other areas of vision research as well as in related scientific disciplines.

Measurement of IOP

The existence of circadian variations in IOP prevents the use of a single tonometric measurement as the basis for deciding whether open-angle glaucoma is present or absent. In fact, more often than not, a diurnal curve will have to be determined for the patient to predict what future course the IOP will take. Jenson and Maumenee suggest that when such a curve cannot be determined or where deterioration of the visual field occurs with apparent good medical control, home monitoring of IOP by a family member of the patient with a disposable tonometer may be useful.[17]

An alternative and potentially more satisfactory approach for round-the-clock IOP measurements is the use of a noninvasive and nonirritative device for constant IOP measurement. An intraocular pressure monitoring contact lens, developed at the University of Utah under an NEI contract, shows promise of offering a means of monitoring intraocular pressure fluctuations for several days at a time. An NEI advisory committee is evaluating the final report of this contract to determine the practicality and applicability of the device for diagnosis and surveillance of glaucoma.[18]

Treatment

As observed earlier in this report, present medical treatment of elevated IOP in primary glaucom relies principally on well-established miotics, epinephrine,

and carbonic anhydrase inhibitors and, in glaucomas secondary to uveitis, on corticosteroids. Where surgery is indicated, laser puncture of the filtration portion of the trabecular meshwork to relieve obstruction of aqueous outflow has received much publicity in recent years. Claims have been made of at least short-lived effectiveness of this technique, particularly with the Q-switched laser.[19] An NEI grant-supported clinical trial of argon laser trabeculotomy is in progress to establish the usefulness of this technique for the relief of excessive IOP in open-angle glaucoma.[20] Another clinical trial is being initiated which will evaluate the benefits of prophylactic iridectomy in angle-closure glaucoma.[21]

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SENSORY AND MOTOR DISORDERS OF VISION

Introduction

The Sensory and Motor Disorders of Vision Program of the National Eye Institute supports clinical and laboratory investigations that bear on disorders of visual information transmission, perceptual synthesis, and oculomotor control. Examples of such disorders are strabismus, amblyopia, degenerations of the optic nerve, and congenital nystagmus. The preponderance of effort supported under this program is related to basic biological research designed to augment knowledge of the structure and function of the visual sensory nervous system and the neural systems controlling eye movement. Substantial support has also been extended to psychophysical investigations, that is, to research on the relationship between the physical parameters of visual stimuli and the visual response of normal persons and those suffering from disorders of vision.

Of the total cases of severe visual impairment in the United States, some 10% derive from sensory-motor disorders of vision.[1] About 3% of the population have manifest strabismus. A much larger percentage have subclinical defects related to disparity of retinal images, e.g. loss of stereopsis or binocular fusion, or functional suppression. The prevalence of amblyopia in the general population is also estimated at 3%. The incidence of optic nerve diseases in the United States population has been set at 53,000 cases per year. Cerebral diseases in the visual areas produce blindness or defective vision in large numbers of persons, particularly the elderly. Congenital and acquired disorders of the oculomotor control system, e.g. abnormalities of gaze, 3rd cranial nerve paralysis, or oculomotor dyslexia result in serious handicap. Refractive myopia, which is of general concern, also falls under the purview of the Sensory and Motor Disorders of Vision program.

A broad array of biological and behavioral disciplines, among them neuroanatomy, neurochemistry, neurophysiology, genetics, bioengineering, biomathematics, human psychophysics and animal behavioristics bring their concepts and techniques to bear on the objectives of this program. Among these objectives are: (1) elucidation of the neural basis of normal and abnormal visual development, (2) tracing of the control system that coordinates the movement of the eyes with each other and with the postural reflexes of the body, and (3) instrumentation for the diagnosis and remediation of visual defects.

Certain approaches have in recent years proven themselves particularly valuable in arriving at the present "state of the art" of visual science. Early monocular or binocular visual deprivation experiments in cats and monkeys provided valuable findings about the morphological and functional changes in the brain that result from abnormal visual experience early in life. These changes may well bear similarities to the abnormal neural substrate in human amblyopia. A search for an "accessory" visual system that functionally complements the classical retino-geniculate-calcarine pathways has yielded scientific dividends. The application of models from pattern recognition technology to the study of human vision has produced new kinds of psychophysical tests with diagnostic potential. Much progress in this past year reflects the dominance of these approaches.

Developmental Studies of Vision

Sherman states the significance of his research program to be the determination of the nature of the interaction between the developing mammalian brain and the visual environment.[2] His research is pursued with neuroanatomical and behavioral methods as well as with single-unit electrophysiological recording. The visual environment is controlled via postnatal lid-suture, enucleation, or controlled illumination in laboratory-reared kittens. Visual orientation and discrimination are studied, as are structural and functional changes in the lateral geniculate nucleus, optic tectum, and striate cortex.

The competitive balance between the two eyes is crucial in disorders such as amblyopia. In several series of experiments, Sherman and Guillery[3] and Wilson, Webb, Sherman[4] aimed at defining the conditions whereby one eye gains a competitive advantage over another in behavioral terms and in terms of the development of central visual corrections. Their tentative conclusion is that an eye can gain competitive advantage only when pattern vision is available. Differences in light or temporal frequencies of stimulation are without effect. Binocular competition was also demonstrated by Wilson and Sherman in the monocularly deprived striate cortex since many simple cells in the deprived monocular segment and very few in the binocular segment were driven normally by the deprived eye.[5] The abnormal complex cells represent a deprivation effect independent of binocular competition since they occur in the monocular segment.

In 1966, Sprague found that a hemianopia produced by a large contralateral posterior cortical lesion in the cat could be partially alleviated by ablation of the superior colliculus contralateral to the cortical lesion.[6] Thus, a visual deficit could be alleviated by an appropriately placed additional lesion. This dramatic phenomenon has been tentatively explained by the supposition that the colliculi normally receive a balanced set of inputs from the two cortices, a balance that is disturbed by an unilateral lesion but in some way restored by the additional ablation. Sherman has recently confirmed and extended these findings and complemented them with stimulation studies.[7] The "Sprague effect" has been found to be a reproducible phenomenon in cats. Visual behavior that can be mediated via retino-tectal pathways can be unmasked if the superior colliculi are functionally disconnected from each other in animals with large cortical lesions. What the specific neuronal correlates of this unmasking are remains to be determined by single-unit electrical recording planned for the future.

In addition to the well-known projections of the vertebrate retina upon the thalamic geniculate nuclei, the retina also connects directly with several other neuronal groupings of the midbrain. Hayhow[8] and Giolli[9] identified a prominent pathway to a group of nuclei referred to as the "accessory optic nuclei" (AON). Recently, Karten has received a National Eye Institute award for the study of the relation between certain retinal ganglion cells in birds and the AON. The study has potential significance because of the discovery by Brauth and Karten that the AON projects directly to the cerebellum, suggesting that a "lemniscal" channel may exist between the avian peripheral retina and the flocculo-modular lobe of the cerebellum which is implicated in the oculovestibular reflexes and the control of eye movements.[10] Should

a homologous channel exist in mammals, particularly humans, the anatomical basis may have been found for a dynamic spatial orientation disjunct from conscious light perception. An NEI Workshop on Research Opportunities Relevant to the Management of Severe Visual Impairment, June 24-25, 1977, identified precisely this topic, i.e. the separation of object recognition and spatial orientation, as an exciting concept in relation to the assessment and remediation of low-vision status. (It is known that humans blind from massive occipital lobe lesions may grasp in the general direction of light or eye movement without conscious experience.) Conceivably, a residual midbrain-cerebellum channel could be exploited in rehabilitation training.

The primary research objectives identified by Karten include: a) identification of those retinal ganglion cells that project to the AON and the characterization of their density, distribution, and dendritic morphology; b) histochemical study of the projection pathway to determine the neurotransmitters present (ACh or catecholamines) as well as the chemical properties of receptors present at the AON; and c) electron microscopic or Golgi studies of retinal axonal arborization. Particularly noteworthy is Karten's intent to characterize the "accessory optic pathway" in neurochemical terms. Success of this endeavor would open the way to pharmacological modification of function in this visual-postural system, with the promise of possible drug treatment of certain neurophthalmological disorders.

Neurophysiological Properties of Retinal Ganglion Cells

Enroth-Cugell and co-workers originally identified two functional classes of retinal ganglion cells in the cat, the X and Y cells.[11] Their X, Y-transient/sustained dichotomy has recently been revised and extended to supra-retinal levels. Tobin, in Enroth-Cugell's laboratory, has studied the temporal frequency characteristics of the center response mechanism of the X and Y retinal ganglion cells.[12] At each of four levels of background illumination (field adaptation) stimulus, conditions were chosen so that responses of the cells to square-wave stimuli showed little or no evidence of surround antagonism. Responses to sinusoidal stimuli of different frequencies (0.2-25 Hz) were then recorded at the same four adaptation levels, and plots of modulation frequency versus (1) sensitivity in absolute terms, (2) contrast sensitivity, and (3) phase angle were compared. These plots showed that only small differences exist between the temporal properties of X and Y cells when driven by their center mechanism. These findings suggest that the interaction of the surround with the center causes the Y cell responses to be more transient than those of X cells. In turn, the Y system appears better suited to process temporal variations than the X system.

A. B. Bonds, working with Enroth-Cugell, has related ganglion cell activity to bleaching adaptation.[13] From the following ganglion cell results on recovery of (rod) threshold after one minute bleaches, it was evident that the quantitative relationship between bleached rhodopsin and ganglion cell threshold cannot be a simple one: (1) regeneration of rhodopsin could not be described by a single exponential during any phase whereas the late phase of ganglion cell threshold recovery could, and the early phase of adaptation seemed to consist of the product of two exponentials; (2) ganglion cell recovery proceeded at the highest rate during the very period when rhodopsin

regeneration was almost halted; and (3) ganglion cell threshold was still above its pre-bleach, dark-adapted value by 0.5-2.3 log units when rhodopsin was completely regenerated at 35 minutes after extinction of the bleaching light.

In related studies, Jakiila has investigated the dependence of retinal ganglion cell sensitivity on movement of the background pattern.[13] From previous investigations, it is well established that the sensitivity of concentric cat retinal ganglion cells to a centered test spot can be reduced by a concentric disk of steady background illumination, the reduction in sensitivity being determined by the effective adapting flux. Experiments by Jakiila and others show that a moving pattern of background illumination can cause a greater reduction in sensitivity than that produced by the same background when stationary, even though the adapting flux is the same. Such findings underline the importance of temporal modulation in the control of ganglion cell activity.

Efferent Control of Eye Sensitivity

Centrifugal pathways from brain to retina have long been known but their function has been obscure. Now, Barlow and co-workers have turned limulus model into an effective tool for studying efferent neural regulation of visual sensitivity.[14] Working with microelectrodes on the lateral eye in situ, they have discovered that it undergoes circadian changes in sensitivity, but such changes occur only if the lateral eye nerve remains intact. When the animal is kept in constant darkness, the lateral eye elicits small-amplitude ERG responses during normal daylight hours and large-amplitude responses during the night. Each ERG response can be elicited by a brief flash of fixed intensity. Prior to initiating this experiment, the animal was placed for several days in an aquarium exposed to exterior lighting conditions, i.e. natural day, night, sunrise, and sunset. On the day of experiment the animal was placed in an aquarium in a light-tight shielded cage, and electrodes and fiber optic stimulators were aligned on one of the lateral eyes. ERG data were collected under constant darkness for two days. On the second day after the experiment began a snare was inserted around the optic nerve trunk through a small hole in the carapace. On day three the snare was pulled to section the optic nerve. No change in ERG could be detected after the optic nerve was cut.

The circadian changes of ERG amplitude observed by Barlow correspond closely to the circadian changes in pigment migration in the retinular cells. These results strongly suggest that efferent axons in the optic nerve trunk innervate the photoreceptor cells of the eye and control their sensitivity by initiating movement of screening pigment in the photoreceptors. Evidence of neurosecretory granules in efferent terminations in the photoreceptors and of a microtubular system for pigment migration lend further support to the notion of efferent control of pigment migration. These results appear to be the only direct evidence obtained to date for efferent control of photoreceptor sensitivity in the limulus eye or in any other visual system.

Mathematical Analysis of the Visual Process

The quantitative understanding of the integrative action whereby the visual system produces a final percept requires, in the first place, formulation of the dynamical laws that interacting visual neurons obey. In the laboratory of Knight and Shapley the dynamics of the three component processes of excitation, lateral inhibition, and self-inhibition have been expressed, particularly in terms of transfer functions. Recently, this group of investigators have undertaken the creation of new stimulus techniques that are used as tools in elucidating visual dynamics.[15] The method uses a stimulus whose variable part is a superposition of a substantial number of sine waves. The essence of the technique is that nonlinearities in the system under stimulation will yield predictable combination frequencies. The stimulus repeats after one minute as soon as it has run through a sample of all phase combinations of its component sine waves.

The set of response frequencies is discrete and thus, in principle, covers zero bandwidth. This allows digital filtering of the output in a way that will reject autonomous wideband noise to an arbitrary degree. In practice, a one minute experimental cycle allows processing the response with a set of parallel narrow-pass digital filters (centered at the input frequencies and at their known combination frequencies), each with a bandwidth of only one-thirtieth Hz. Each of these filters is also so constructed that it has a set of strict nulls placed at all the other output frequencies in the discrete response spectrum. Thus, one can separate cleanly the nonlinear part of the response from the linear part while strongly rejecting autonomous noise.

A general theory of nonlinear system response to any given stimulus ensemble has been developed. The general theory gives an explicit description of how the system's response to any given stimulus ensemble leads to a standard, straightforward dynamical characterization. (The characterization is in terms of the well-known "Volterra series" of homogeneous symmetric functionals of ascending order over the past history of the stimulus signal.) The stimulus ensemble of a discrete set of superimposed sine waves leads easily to a simple final result. A fairly general type of ensemble which consists of "colored" Gaussian noise likewise leads to a simple general result. A limiting case is that of infinite-bandwidth "white" noise, which retrieves the Wiener description whereas the opposite limit of "zero bandwidth" noise with a discrete spectrum retrieves the description for the stimulus ensemble.

Analytic methods of this sort may well extend across the disciplines of unit-cell neurophysiology, evoked visual potentials, and human psychophysics. The potential for such interdisciplinary developments will be encouraged by NEI.

Clinical Psychophysics

At present, much of visual testing and assessment in the clinic depends upon the skill and experience of the examiner as well as the verbal response of patients. These subjective procedures are further influenced by uncontrolled

physical, psychological and environmental variables that confound the evaluation of a patient's condition and the comparison of diagnostic groups.

A recent workshop sponsored by the NEI, "The Role of Psychophysics and Physiological Optics of Ophthalmic Diagnosis and Patient Evaluation"[16] focused on the challenges faced by psychophysical science in relation to ophthalmic clinical science. The participants agreed that psychophysicists and opticists now have new techniques available to characterize visual functions with greater objectivity and precision. In their view, a need exists to bring these techniques to bear on such problems as localization of anomalies in glaucoma, senile macular degeneration, amblyopia, degenerations of the optic pathway, cerebral lesions, strabismus and disorders of oculomotor control. New approaches to clinical testing using modern psychophysical techniques or optical instrumentation were identified at the workshop.

1. Measurements of the Westheimer effect demonstrate the influence of the size of a surrounding field on the ability to detect a test flash. This technique may be used to localize pathology in the choroid/pigment epithelium, receptor layer, inner and outer plexiform layer, or optic nerve. Such studies by Enoch, Johnson and Fitzgerald open the possibility of pinpointing retinal and neural lesions in a manner beyond the scope of traditional tests of acuity.[17]

2. Visual spatial and temporal modulation transfer functions (contrast grating sensitivity) tests, based on mathematical models of the transfer characteristics of the visual system, may address questions of pathology in the CNS as well as abnormalities in retinal receptive fields.

3. Cortical evoked response measurement of visual acuity will permit objective clinical testing with non-invasive techniques in cases of amblyopia or other sensory disorders in children.

4. Random dot tests of stereopsis, based on global rather than local visual cues, may more adequately evaluate binocularity in cases of strabismus.

5. Tests of infant vision based on preferential looking or operant conditioning techniques may assist in the acquisition of normative data on infant populations.

6. Tests for the assessment of color defects in functional disorders of the visual brain may help determine the severity and course of disease or intoxication.

7. Infrared and other modern electro-optical eye movement recording technology has the potential to complement traditional nystagmography and electromyography. The latter may require uncomfortable electrode implants or may be incapable of resolving very small eye movements for neurophthalmological diagnosis. Several new eye-trackers have potential for application to the detection and characterization of eye movement disorders in the eye clinic.

Recent Initiatives

To stimulate further research on these and all other promising psychophysical and optical tools and to facilitate translation of technical advances into improved ophthalmic diagnosis and treatment, special initiatives were undertaken by the National Eye Institute this past year. One of these was the preparation and publication of two announcements inviting the submission of worthy research proposals related to the application of psychophysical tests to the diagnosis of human visual disorders and to the rehabilitation of the severely visually-impaired.

Another initiative was the funding of clinically imaginative new projects. For example, Bodis-Wollner, who has been among the first to apply contrast grating sensitivity functions to the localization of neurological lesions, received NEI support for a study of sensitivity of neurophthalmological patients to temporally-modulated contrast grating.[18] Bodis-Wollner hopes to use his techniques to distinguish psychophysically between pathology in "transient" and "sustained" pathways in the human visual system.

A third initiative was the award of a special multi-institutional training grant, "Clinical Application of Visual Science", to the University of Florida, Gainesville. Enoch, the program director, will train individuals in psychophysics or physiological optics to apply their science more effectively to clinical ophthalmic problems. It is planned that the trainees will spend approximately one year in each of two or three institutions (for example, U. of Florida, Gainesville, U. of Washington, Seattle, U. of California, Berkeley) in order to achieve the desired training and clinical experience.

The nature of this novel training program is well described by the hypothetical example of a trainee interested in infant vision. Such an individual might start training in behavioral testing methods. After one year, the trainee would move to another institution or preceptor to learn electrophysiological techniques. For a third year, the trainee might go to a clinic where procedures are used to correct the vision of infants with sensory deprivation abnormalities. The training program will be advertised by brochure, and qualified candidates with training in psychophysics, electrophysiology, physiological optics and other relevant disciplines will be considered for admission.

Finally, technology transfer was promoted by an award to H. D. Crane, Stanford Research Institute, Palo Alto, California for the further development of the Purkinje three-dimensional eye-tracker (Cornsweet and Crane[19]) for ophthalmic application. This instrument, originally conceived for the U. S. space program, has proved useful in image stabilization, tracking of the state of accommodation, and nystagmography. A potential exists for its use in the diagnosis of oculomotor disease, in clinical psychophysics, and even in retinal surgery. A workshop to discuss the clinical applicability of such an instrument is planned for the near future. Some twenty research laboratories or clinics now possess the instrument; many have NEI support. A "users group" has been established to foster cooperative research and to provide the Stanford Research Institute with systematic feedback about the instrument's

performance and with suggestions for its further development. A "users group" workshop to discuss these issues is being planned.

Orthokeratology Clinical Trial

Myopia is a condition that affects a very large part of our population. While the etiology of nearsightedness is far from clear, "treatment" in the form of corrective lenses is traditional. In recent years it has been noted by Miller[20], Rengstorff[21] and many others that corneal curvature may be changed by contact lens wear and that consequently the refractive error in vision may also be altered. Since such changes may persist, some practitioners have attempted to exploit the changes in corneal curvature induced by shaped contact lenses to reduce refractive error permanently, a procedure known as "orthokeratology".[22] Orthokeratology, however, has remained unvalidated and controversial.

During fiscal year 1976, NEI received an application from Polse[23], School of Optometry, University of California, Berkeley, requesting grant support for a controlled clinical trial of the effectiveness of orthokeratology. After a positive concept review by an NIH initial review group and concurrence by the National Advisory Eye Council, a conditional award was made for the development of a manual of operations for a randomized, masked clinical trial. The manual of operations subsequently developed was evaluated by NEI staff and external consultants for soundness of experimental design, adequacy of optical measures, attention to side-effects, and ethical safeguards, including informed consent.

At the present time, the manual of operations is undergoing final revisions. The measurements to be undertaken include corneal thickness, refractive error, biological changes in the cornea and visual acuity. The study proposed is a "two-arm" comparison of orthokeratological vs. traditional contact lens therapy. Patient assignment to either treatment is to be at random. Ophthalmologic and optometric examination is to be continuous. An award for actual initiation of the clinical trial is expected to be made soon.

Workshop on General Anesthesia and Visual Receptive Fields

Ethical considerations preclude NEI grantees from using paralyzed, unanesthetized animals in electrophysiological experiments. These restrictions, however, pose difficulties in the mapping of receptive fields, particularly those of cells in the higher divisions of the visual system, which are quite labile. To help overcome these obstacles, a meeting has been planned in fiscal year 1978 of vision researchers active in this field. Some main topics for discussion include: (1) the effect of various anesthetic agents on the receptive fields in the lateral geniculate nucleus, visual cortex, etc., (2) the types of receptive field properties most affected by various anesthetics, and (3) alternatives to paralysis for controlling eye movements. Recommendations to the National Advisory Eye Council on areas of future research need and opportunity pertaining to the above as well as other topics will be formulated at the workshop.

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